10/524434

PCT/DK03/00542

PCT/DK 03/00542

PCT/DK03/00542

REC'D 16 SEP 2003
WIPO PCT

Kongeriget Danmark

Patent application No.: PA 2003 00987

Date of filing: 27 juni 2003

Applicant: (Name and address)
Leukotech A/S
Fruebjergvej 3
DK-2100 København Ø
Denmark

Title: Bactericidal, anti-apoptotic, pro-inflammatory and anti-inflammatory peptides of heparin-binding protein (HBP)

IPC:

This is to certify that the attached documents are exact copies of the above mentioned patent application as originally filed.

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Patent- og Varemærkestyrelsen
Økonomi- og Erhvervsministeriet

9 September 2003

Pia Høybye-Olsen

BEST AVAILABLE COPY

PATENT- OG VAREMÆRKESTYRELSEN

Bactericidal, anti-apoptotic, pro-inflammatory and anti-inflammatory peptides of heparin-binding protein (HBP)

Technical field of the invention

The present invention relates to providing peptides derived from the sequence of heparin-binding protein (HBP) and/or human neutrophil elastase and using said peptides for the manufacture of a medicament for the treatment of Gram positive and/or Gram negative infections, sepsis, disseminated intravascular coagulation, modulation of inflammatory response, and/or prevention of cell apoptosis.

Background of the invention

A local infection or injury in any tissue rapidly attracts white blood cells into the affected region as part of the inflammatory response, which helps fight the infection or heal the wound. The inflammatory response is complex and is mediated by a variety of signalling molecules produced locally by different types of cells. Some of these molecules act on nearby capillaries, causing the endothelial cells to adhere less tightly to one another but making their surfaces adhesive to passing white blood cells. Other molecules act as chemoattractants for specific types of blood cells, such as monocytes, causing these cells to become polarised and crawl toward the source of the attractant.

White blood cells, specifically polymorphonuclear leukocytes (PMNs), produce a large variety of peptides involved in the inflammatory response. Among these peptides is the heparin-binding protein (HBP), which was first isolated from azurophile granules of human PMNs. A highly homologous peptide was also isolated from PMNs of porcine origin and has been named porcine heparin-binding protein (pHBP) (Flodgaard et al., 1991, Eur. J. Biochem. 197: 535-547; Pohl et al., 1990, FEBS Lett. 272: 200 ff.) HBP has otherwise been termed CAP37 (WO 91/00907, US 5,456,674 and 6,484,885) and azurocidin (Wilde et al. 1990, J. Biol. Chem. 265:2038-41).

Sequence analysis of HBP has revealed that the protein bears many similarities to serine proteases, which are important in inflammatory processes, e. g. neutrophil

elastase (47% homology) or protease 3 (43% homology), however HBP lacks protease activity due to mutations of two of three amino acids in the highly conserved catalytic triad. The structure of HBP appears from WO 89/08666 and Flodgaard et al., 1991 (Eur. J. Biochem. 197: 535-547).

HBP was originally studied because of its antibiotic and lipopolysaccharide binding properties (Gabay et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:5610-5614 and Pereira et al., 1993, Proc. Natl. Acad. Sci. USA 90: 4733-7). However, a number of experimental evidence now supports the concept that HBP is a multifunctional protein, and, in addition to its bactericidal role, is involved during the progression of inflammation due to its effect on the recruitment and activation of monocytes (Pereira et al., 1990, J. Clin. Invest. 85:1468-1476, and Rasmussen et al., 1996, FEBS Lett. 390:109-112), recruitment of T cells (Chertov et al., 1996, J. Biol. Chem. 271:2935-2940), as well as on the induced contraction of endothelial cells and fibroblasts (Ostergaard and Flodgaard, 1992, J. Leuk. Biol. 51:316-323). Ostergaard and Flodgaard (op. cit.) also disclose increased survival of monocytes treated with HBP. Furthermore, in animal models of fecal peritonitis, HBP treatment has been shown to rescue mice from an otherwise lethal injury (Mercer-Jones et al., 1996, In: Surgical Forum, pp. 105-108; Wickel et al., 1997, In: 4th International Congress on the Immune Consequences of Trauma, Chock and Sepsis, Munich, Germany, pp. 413-416).

Using synthetic peptides derived from the sequence of human HBP in laboratory and preclinical research some functions of the protein has been structurally localised within the molecule of HBP. Thus, it has been shown that, for example, a high Gram negative bactericidal activity of human HBP is most probably associated with residues 20-44 of the human HBP amino acid sequence (Pereira et al., 1993, Proc. Natl. Acad. Sci. USA 90: 4733-7 and US 6,107,460). The amino acid residues 95-122 of the human HBP sequence have been associated with a capacity of the protein to stimulate protein kinase C in vascular endothelial cells (Pereira et al., 1996, J. Leukoc. Biol. 60:415-22).

It would be advantageous to produce new peptides derived from the sequence of human HBP, porcine HBP, or analogues of these sequences, such as, for example, neutrophil elastase, to use for the manufacture of new bactericidal, anti-apoptotic

medicaments and medicaments for modulation of an inflammatory response, especially the inflammatory response to bacterial infection.

Summary of the invention

Thus, in one embodiment the present invention relates to providing peptides having a peptide having a sequence of at most 44 amino acid residues comprising a motif of the formula

X^1-X^2-Cys-X^3-X^4-X^5-X^6-X^7-X^8-X^9-X^{10}-X^{11}-X^{12}-X^{13}-X^{14}-X^{15}-X^{16}-X^{17}-Cys-X^{18}-X^{19},

wherein
the side chains of the two Cys residues are connected via a disulfide bond,
wherein
X can be an amino acid sequence or a single amino acid residue selected either from Group 1 consisting of Ala, Gly, and Ser,
Group 2 consisting of Arg and Lys,
Group 3 consisting of His, Ile, Leu, Met, Phe, Pro, Thr, Val, Trp, and Tyr,
Group 4 consisting of Asn and Gln , or
Group 5 consisting of Ala, Asn, Arg, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val,
wherein
X^1 can be represented by a sequence consisting of 2-5 amino acid residues or an amino acid residue selected from Group 2;
X^2 is selected from Group 5 or Group 3;
X^3, X^{15} and X^4 are selected from Group 1;
X^5 is Thr or selected from from Group 1;
X^6, X^{11}, X^{12}, X^{13} and X^7 are selected from Group 3;
X^8 and X^{17} are selected from Group 1, 3 or 4;
X^9 is selected from from Group 5, 1 or 3;
X^{10} is selected from from Group 2, 3 or 4;
X^{14} is Ser or selected from from Group 3;
X^{16} and X^{18} is selected from Group 1 or 3
X^{19} can be represented by a sequence consisting of 2-5 amino acid residues or a single amino acid residue selected from Group 5, 2, or 4,

with the proviso, that when X^1 includes Pro, then X^{19} is Gln.

The invention concerns providing peptides derived from the sequence of human HBP (hHBP) and/or porcine HBP (pHBP) and/or human neutrophil elastase capable of proinflammatory or anti-inflammatory activity, bactericidal and/or monocyte attractive activity, and/or capable of preventing cell apoptosis.

Further, the present invention discloses a recombinant process for the production of the above peptides, and the use of the peptides of the invention for the manufacture of a medicament for prevention or treatment of Gram negative and/or Gram positive bacterial infections, sepsis, severe sepsis, septic shock, disseminated and/or intravascular coagulation, stimulation or inhibition of the inflammatory response, or cell apoptosis.

Additionally, the invention related to use of an antibody directed against an epitope comprising one or more of the sequences set forth in SEQ ID NO: SEQ ID NOS: 12-39, 233-253, 395-421, said antibody is capable of inhibiting the inflammatory response associated with one or more biological activities of hHBP or a hHBP fragment comprising amino acids 20-44 according to the sequence identified as SEQ ID NO: 1, for the manufacture of a medicament for pathological conditions wherein inhibition of inflammatory response is required, and to use of an antibody directed against an epitope comprising one or more of the sequences set forth in SEQ ID NO: 2-11, 40-232, 254-394, 421-587, said antibody is capable of stimulating the inflammatory response associated with one or more biological activities of hHBP or a hHBP fragment comprising amino acids 20-44 according to the sequence identified as SEQ ID NO: 1, for the manufacture of a medicament for pathological conditions wherein stimulation of inflammatory response is required

Figures

Figure 1 depicts IL-6 secretion induced by HBP peptides in the absence of bacterial components.

Figure 2 shows the effect of HBP 20-44 peptides on LPS induced IL-6 secretion

Figure 3 shows the effect of HBP 20-44 peptides on LPS induced IL-6 secretion in the presence of PGN.

Figure 4 shows the effect of HBP 20-44 peptides on LPS induced IL-6 secretion in the presence of PCW.

Figure 5 shows the effect of N-Ac, C-amido hHBP 20-44 on PGN induced IL-6 secretion.

Figure 6 shows the effect of R34Q pHBP 20-44 on PGN induced IL-6 secretion.

Figure 7 shows the effect of different substitutions in the sequence of hHBP and p HBP on production of IL-6 induced by LPS.

Figure 8 shows the effect of different substitutions in the sequence of hHBP and p HBP on production of IL-6 induced by PGN.

Table 1 shows the potential applications for mono-functional peptides of the invention.

Detailed description of the Invention

Inflammation

The present invention relates to providing peptides and using said peptides for the manufacture of a medicament for modulation of the inflammatory response.

Inflammation is a defence reaction caused by tissue damage due to a mechanical injury or bacterial, virus or other organism infection. The inflammatory response involves three major stages: first, dilation of capillaries to increase blood flow; second, microvascular structural changes and escape of plasma proteins from the bloodstream; and third, leukocyte transmigration through endothelium and accumulation at the site of injury and infection. The inflammatory response begins with a release of inflammatory mediators. Inflammatory mediators are soluble, diffusible molecules that act locally at the site of tissue damage and infection, and at more distant sites,

influencing consequent events of the inflammatory response. Inflammatory mediators can be exogenous, e. g. bacterial products or toxins, or endogenous, which are produced within the immune system itself, as well as injured tissue cells, lymphocytes, mast cells and blood proteins.

In one aspect the present invention relates to the inflammatory response to bacterial infection.

By "bacterial infection" in the present context is meant the invasion of normally sterile host tissue by bacteria. Bacterial infection of the invention may be due to invasion of either Gram negative or Gram positive bacteria, or a combination thereof or other infectious agents including fungi and virus. In one embodiment the present invention relates to the inflammatory response due invasion of Gram negative bacteria selected from the group comprising Acetobacteriaceae, Alcaligenaceae, Bacteroidaceae, Chromatiaceae, Enterobacteriaceae, Legionellaceae, Neisseriaceae, Nitrobacteriaceae, Pseudomonadaceae, Rhizobiaceae, Rickettsiaceae, Spirochaetaceae, Vibrionaceae, Brucella, Chromobacterium

In another embodiment the invention relates to the inflammatory response due to invasion by Gram positive bacteria selected from the group comprising Bacillaceae, Micrococcaceae (for example Staphylococcus aureus), Mycobacteriaceae (for example Staphylococcus pneumoniae), Peptococcaceae.

In an additional another embodiment the invention relates to the inflammatory response associated with sepsis, severe sepsis and/or septic shock.

By "sepsis" in the present context is meant the systematic inflammatory response to bacterial infection, characterised by one or more of the following conditions as a result of infection: temperature >38 °C or < 36 °C, heart rate > 90 beats/min, respiratory rate > 20 breaths/min or $PaCO_2$ < 32 torr (< 4.3 kPa), and WBC > 12 000 cells/mm^3 or < 4000 cells/ mm^3 or 10% immature (band) forms.

By "severe sepsis" in the present context is meant sepsis associated with organ dysfunction, hypoperfusion, or hypotension. hypoperfusion and hypotension abnor-

malities may include, but are not limited to, lactic acidosis (acidic condition in blood), oliguria (meaning reduction in urine production), or acute alteration in mental status.

By "septic shock" in the present context is meant sepsis with hypotension despite adequate fluid resuscitation, along with the presence of perfusion abnormalities that may include, but are not limited to, lactic acidosis, oliguria, or acute alteration in mental status.

In yet another embodiment the invention relates to the inflammatory response associated with disseminated intravascular coagulation (DIC).
By "DIC" in the present context is meant a pathophysiologic condition involving a continuum of events that occur in the coagulation pathway in association with a variety of well-defined clinical situations, including sepsis, major trauma, and abruptio placenta, and with laboratory evidence of the following: procoagulant activation, fibrinolytic activation, inhibitor consumption and biochemical evidence of end-organ damage or failure.

Proinflammatory peptides and antibodies

It is an objective of the present invention to provide new peptides, which are capable to serve as additional mediators of the inflammatory response, the so-called pro-inflammatory peptides are particularly useful but not limited to patients selected from groups of immune-suppressed patients, cancer patients, patients with autoimmune diseases and patients undergoing major surgery.

In the present context by the term " pro-inflammatory peptide" is meant an artificial peptide compound which is capable of

i) Stimulating, either alone or in synergistic action with bacterial products including, but not limited to LPS (Lipopolysaccharide), PGN (peptidoglycan), LTA (Lipotechoic acid), MDP (muramyldipeptide) and PCW (purified cell wall from bacteria), the gene expression in the immune cells, preferably monocytes/macrophages, leading to secretion of endogenous inflammatory mediators including receptors for inflammatory mediators and transcription factors involved in the signal tranduction of the inflammatory mediators, said mediators being preferably selected from the group comprising cytokines,

selected from the group TNFalpha IL-1, IL-6, G-CSF, GM-CSF,M-CSF. Chemokines selected from the group comprising IL-8, MCP-1, receptors selected from the groupTissue factor and IL-2Ralpha. and/or

ii) activating the production of bradykinin by the phase contact system, and/or;

iii) serving as an attractant for monocytes, and/or

iv) increasing the life-time of monocytes, neutrophils and other immune cells serving as an inhibitor of apoptosis, and/or

v) activating vascular endothelial cells to express the adhesion molecules, said adhesion molecules being preferably selected from the group comprising PECAM, ICAM-1, E-selectins, VCAM-1, and/or

vi) activate the contact phase system to produce bradykinin leading to an increased vascular permeability, and/or

vii) increase the phagocytic potential of monocytes/macrophages, and/or

viii) upregulate class-II MHC.

In one embodiment the pro-inflammatory peptide of the invention is a peptide

i) derived from any of the sequences set forth in SEQ ID NO: 1, SEQ ID NO:588, or SEQ ID NO: 589,

ii) comprising one or more of the sequences set forth in SEQ ID NO: 2-587,

iii) capable of at least one of the above activities (i-viii) of an pro-inflammatory compound, more preferable at least two of the above activities, even more preferable at least tree of the above activities, even more preferable at least four of the above activities, even more preferable at least five of the above activities, even more preferable at least six of the above activities, even more preferable at least seven of the above activities, and most preferably eight of the above activities.

In the present context the term "synergistic action" refers to the situation where the combined action of a bacterial product and a peptide of the present invention is a stronger pro-inflammatory stimulant than the pro-inflammatory stimulant a bacterial product or the present peptide, respectively would be on their own.

In another embodiment the invention provides a pro-inflammatory peptide capable of stimulating either alone or in synergistic action with bacterial products including, but not limited to LPS (Lipopolysaccharide), PGN (peptidoglycan), LTA (Lipotechoic

acid), MDP (muramyldipeptide) and PCW (purified cell wall from bacteria) the secretion of cytokine IL-6 from monocytes, comprising two or more sequences set forth in SEQ ID NOS: 15-36, wherein said sequences constitute a contiguous sequence derived from the sequence of hHBP set forth in SEQ ID NO:1. Further, the pro-inflammatory peptide may be used for the manufacture of a medicament for the treatment of individuals having suppressed immune system, cancer, auto-immune diseases and/or trauma.

In a preferred embodiment the invention concerns an pro-inflammatory peptide, wherein said peptide has the sequence NQGRHFCGGALIHARFVMTAASCFQ (SEQ ID NO: 594). Even more preferred the peptide, wherein the sequence identified in SEQ ID NO: 594 has the N-terminal and C-terminal modified, as for example the N-terminal amino group being amidated and the C-terminal carboxy group being acetylated.

The invention also relates to a pro-inflammatory antibody.

The pro-inflammatory antibody of the invention is an antibody, which is

i) capable to recognise an epitope comprising one or more of the sequences set forth in SEQ ID NO: 2-11, 40-232, 254-394, 421-587,

ii) capable of stimulating the inflammatory response associated with one or more biological activities of hHBP or a hHBP fragment comprising amino acids 20-44 according to the sequence identified as SEQ ID NO: 1,

iv) capable of at least one of the above activities (i-viii) of an pro-inflammatory compound, more preferable at least two of the above activities, even more preferable at least tree of the above activities, even more preferable at least four of the above activities, even more preferable at least five of the above activities, even more preferable at least six of the above activities, even more preferable at least seven of the above activities, and most preferably eight of the above activities.

The pro-inflammatory antibody of the invention may be either a polyclonal or monoclonal antibody. In a preferred embodiment the pro-inflammatory antibody of the invention is a monoclonal antibody. In a more preferred embodiment the pro-inflammatory antibody of the invention is capable to stimulate production of cytokine

IL-6. In the most preferred embodiment the pro-inflammatory antibody is the antibody F17A5B1.

Anti-inflammatory peptides and antibodies

It is another important objective of the invention to provide new anti-inflammatory peptide, which are capable of serving as inhibitors of the sustained inflammatory response.

The continuous presence of inflammatory mediators, such as for example TNF alpha in the body in response to sustained presence of bacterial products or even live bacteria locally during days or weeks following trauma and/or infection promotes the reactions to inflammation, such as, for example, heat, swelling, and pain. The sustained inflammatory response has been proven to be very harmful to the body. If the bacterial products or live bacteria become spread universally in the body from their local focus the inflammatory reaction becomes overwhelming and out of control and leads to sepsis which eventually progress further to severe sepsis and septic shock. Anti-inflammatory peptides may be used to block or suppress the overwhelming sustained inflammatory response represented by a massive and harmful cytokine cascade in the blood and vital organs such as lung, liver intestine, brain and kidneys.

In the present context by the term "anti-inflammatory compound" is meant a compound which is capable of

i) decreasing or inhibiting the gene expression in the immune cells, preferably monocytes/macrophages in response to bacterial products, live bacteria or trauma to produce endogenous inflammatory mediators including receptors for inflammatory mediators and transcription factors involved in the signal transduction of the inflammatory mediators, said mediators being preferably selected from the group comprising cytokines, selected from the group TNFalpha IL-1, IL-6, G-CSF, GM-CSF, M-CSF. Chemokines selected from the group comprising IL-8, MCP-1, receptors selected from the group Tissue factor and IL-2Ralpha. and/or

ii) decrease or inhibit the production bradykinin by the phase contact system, and/or;

iii) decrease or inhibit the attractant potential for monocytes, and/or

iv) decrease or inhibit the life-time of monocytes, neutrophils and other immune cells serving as an inducer of apoptosis, and/or

v) decrease or inhibit vascular endothelial cells to express the adhesion molecules, said adhesion molecules being preferably selected from the group comprising PECAM, ICAM-1, E-selectins, VCAM-1 and/or

vi) decrease or inhibit activation of the contact phase system to produce bradykinin leading to increased vascular permeability, and/or

vii) stimulate the synthesis of an anti-inflammatory mediator selected from the group of IL-10 and IL-12, and/or

viii) removing endotoxin from septic patients, and/or

In one embodiment the anti-inflammatory peptide compound of the invention is a peptide

i) derived from any of the sequences set forth in SEQ ID NO: 1, SEQ ID NO: 588, or SEQ ID NO: 589,

ii) comprising one or more of the sequences set forth in SEQ ID NO: 2-587,

iii) capable of at least one of the above activities of an anti-inflammatory compound, more preferable at least two of the above activities, even more preferable at least tree of the above activities, even more preferable at least four of the above activities, even more preferable at least five of the above activities, even more preferable at least six of the above activities, even more preferable at least seven of the above activities, even more preferable at least eight of the above activities, and most preferably nine of the above activities.

In another embodiment the invention provides an anti-inflammatory peptide capable of inhibiting the secretion of cytokine IL-6 from monocytes in response to bacterial products including, but not limited to, LPS (Lipopolysaccharide), PGN (peptidoglycan), LTA (Lipotechoic acid), MDP (muramyldipeptide) and PCW (purified cell wall from bacteria), comprising two or more sequences set forth in SEQ ID NOS: 233-253, wherein said sequences constitute a contiguous sequence derived from the sequence of pHBP set forth in SEQ ID NO:588.

In a preferred embodiment of the invention an inflammatory peptide has the sequence KQGRPFCAGALVHPRFVLTAASCFR (SEQ ID NO: 593). Even more

preferred the peptide, wherein the sequence identified in SEQ ID NO: 593 has the N-terminal and C-terminal modified, as for example the N-terminal amino group being amidated and the C-terminal carboxy group being acetylated.

The invention also relates to an anti-inflammatory antibody.

The anti-inflammatory antibody of the invention is an antibody, which is

iii) capable to recognise an epitope comprising one or more of the sequences set forth in SEQ ID NO: 12-39, 233-253, 395-421,

iv) capable of inhibiting the inflammatory response associated with one or more biological activities of hHBP or a hHBP fragment comprising amino acids 20-44 according to the sequence identified as SEQ ID NO: 1,

v) capable of at least one of the above activities (i-viii) of an anti-inflammatory compound, more preferable at least two of the above activities, even more preferable at least tree of the above activities, even more preferable at least four of the above activities, even more preferable at least five of the above activities, even more preferable at least six of the above activities, even more preferable at least seven of the above activities, and most preferably eight of the above activities.

The anti-inflammatory antibody of the invention may be either a polyclonal or monoclonal antibody. In a preferred embodiment the anti-inflammatory antibody of the invention is a monoclonal antibody. In a more preferred embodiment the anti-inflammatory antibody of the invention is capable to inhibit production of cytokine IL-6. In the most preferred embodiment the pro-inflammatory antibody is the antibody F17A5B4.

Peptides

It is an objective of the present invention to provide one or more peptides for the manufacture of a medicament for prevention and/or treatment of Gram positive and/or Gram negative infections, sepsis, severe sepsis, septic shock and/or disseminated intravascular coagulation, and/or for modulation of inflammatory response, and/or prevention of cell apoptosis.

In one embodiment the invention concerns a peptide having a sequence of at most 44 amino acid residues comprising a motif of the formula

X^1-X^2-Cys-X^3-X^4-X^5-X^6-X^7-X^8-X^9-X^{10}-X^{11}-X^{12}-X^{13}-X^{14}-X^{15}-X^{16}-X^{17}-Cys-X^{18}-X^{19},

wherein

the side chains of the two Cys residues are connected via a disulfide bond,

wherein

X can be an amino acid sequence or a single amino acid residue selected either from Group 1 consisting of Ala, Gly, and Ser,

Group 2 consisting of Arg and Lys,

Group 3 consisting of His, Ile, Leu, Met, Phe, Pro, Thr, Val, Trp, and Tyr,

Group 4 consisting of Asn and Gln , or

Group 5 consisting of Ala, Asn, Arg, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val,

wherein

X^1 can be represented by a sequence consisting of 2-5 amino acid residues or an amino acid residue selected from Group 2;

X^2 is selected from Group 5 or Group 3;

X^3, X^{15} and X^4 are selected from Group 1;

X^5 is Thr or selected from from Group 1;

X^6, X^{11}, X^{12}, X^{13} and X^7 are selected from Group 3;

X^8 and X^{17} are selected from Group 1, 3 or 4;

X^9 is selected from from Group 5, 1 or 3;

X^{10} is selected from from Group 2, 3 or 4;

X^{14} is Ser or selected from from Group 3;

X^{16} and X^{18} is selected from Group 1 or 3

X^{19} can be represented by a sequence consisting of 2-5 amino acid residues or a single amino acid residue selected from Group 5, 2, or 4,

with the proviso, that when X^1 includes Pro, then X^{19} is Gln.

In a preferred embodiment X^1 in sequence of the peptide of the invention is represented by an amino acid sequence selected from SEQ ID NOS: 607-612. In another preferred embodiment X^1 is Arg. Another preferred embodiment for the sequence of the peptide is Phe in position X^2. It is also preferred Ala or Gly as the X^3 residue. The peptide preferably has Gly as the X^4 residue. The X^5 residue is preferably represented by Ala, and X^6 by Leu. The X^7 may preferably be selected

from the group containing Ile, Leu, Met or Val. The peptide according to another preferred embodiment has X^8 represented by His or Val. The position X^9 in the sequence of the peptide may preferably be occupied by a residue selected from Ala, Phe or Pro. X^{10} is preferably represented by Arg, and X^{11} is Phe or Pro. The position X^{12} is preferably occupied by His or Val., and X^{13} is preferably selected from the group consisting Ile, Leu, Met or Val. The peptide has preferably Thr in position X^{14}, Ala in position X^{15} and X^{16}, and X^{17} is preferably Ser. The X^{18} residue is preferably represented by Phe. X^{19} in one preferred embodiment is represented by a sequence identified as SEQ ID NO: 613, and in another preferred embodiment is represented by Arg or Gln.

Another preferred embodiment of a peptide having the sequence comprising the above motif is that N-terminal and/or C-terminal of said peptide may be modified. Thus, the C-terminal carboxy group in a more preferred embodiment is amidated, and the N-terminal in another more preferred embodiment is acetylated.

Furthermore, the invention in the other preferred embodiments concerns particular amino acid sequences comprising the motif disclosed above. Thus, the peptide according to the present invention preferably has a sequence selected from
KQGRPFCAGALVHPRFVLTAASCFR (SEQ ID NO: 593),
NQGRHFCGGALIHARFVMTAASCFQ (SEQ ID NO: 594),
KQGRHFCGGALIHARFVMTAASCFR (SEQ ID NO: 595),
KQGRPFCGGALIHARFVMTAASCFR (SEQ ID NO: 596),
KQGRHFCGGALIHPRFVMTAASCFR (SEQ ID NO: 597),
KQGRPFCGGALIHPRFVMTAASCFR (SEQ ID NO: 598),
RFCSAATLVFRPHVLAGACFPRGQK (SEQ ID NO: 599),
NQGRPFCAGALVHPRFVLTAASCFR (SEQ ID NO: 600),
KQGRPFCAGALVHPRFVLTAASCFQ (SEQ ID NO: 601),
NQGRPFCAGALVHPRFVLTAASCFQ (SEQ ID NO: 602),
KQGRPFCAGALVHPQFVLTAASCFR (SEQ ID NO: 603),
LRGGHFCGATLIAPNFVMSAAHCVA (SEQ ID NO: 604),
RRGGHFCGATLIARNFVMSAVHCVN (SEQ ID NO: 605) and
RSREYRCGGTLVSQRYILTAASCAA (SEQ ID NO: 606).

The sequences identified in SEQ ID NO: 593 and 594 according to the invention are preferably modified in the N- and C-terminus as described above.

All peptides of at most 44 amino acids length comprising the motif as above are in the scope of the present invention. However, the invention preferably concerns peptides wherein the positions X^{15} -X^{16}- X^{17} in the motif are Ala-Ala-Ser correspondingly, in positions X^{1} to X^{14} and X^{18} to X^{19} may be any amino acid residues connected in a contiguous polypeptide chain. The preferred embodiments for the positions are as described above. Moreover, it is further preferred, if the peptide comprises one or more amino acid sequences are selected from SEQ ID NOS: 12-39. Another preferred embodiment if the sequence comprises one or more amino acid sequences are selected from SEQ ID NOS: 233-253. It is still another preferred embodiment if the peptide comprises one or more amino acid sequences are selected from SEQ ID NOS: 233-253 and SEQ ID NO: 34. In yet preferred embodiment the peptide comprises one or more amino acid sequences selected from SEQ ID NOS: 233-253 and SEQ ID NO: 21. Still yet preferred embodiment is a peptide comprising one or more amino acid sequences are selected from SEQ ID NOS: 233-253 and SEQ ID NO: 21 and SEQ ID NO: 34. Also preferred a peptide comprising one or more amino acid sequences are selected from SEQ ID NOS: 395-421.

The sequences of the peptide may be derived from any longer polypeptide sequence of natural or artificial origin. However the invention concerns some preferred embodiments for such the sequence. In a first preferred embodiment the invention concerns one or more amino acid sequences which are derived from the sequence of human heparin-binding protein (hHBP) set forth in SEQ ID NO: 1. In another preferred embodiment the invention concerns one or more amino acid sequences derived from the sequence of porcine heparin-binding protein (pHBP) set forth in SEQ ID NO: 588. In still another preferred embodiment the invention concerns one or more amino acid sequences derived from the sequence of human neutrophil elastase set forth in SEQ ID NO: 589.

Additionally, a preferred peptide comprising the above motive according to the invention comprises one or more amino acid sequences set forth in SEQ ID NOS: 2-587.

A peptide as described above according to the invention is capable of inhibiting the secretion of cytokine IL-6 from monocytes.

It is another objective of the present invention to produce peptides as small as possible, yet exhibiting the desired effect(s).

In one embodiment, the invention relates to providing a peptide consisting of at most 8 amino acids comprising one or more of the amino acid sequences set forth in SEQ ID NOS: 2-587.

In another embodiment, the invention relates to providing a peptide consisting of at most 12 amino acids comprising one or more of the amino acid sequences set forth in SEQ ID NOS: 2-14, 22-36, 46-107, 115-185 and 195-587.

In still another embodiment, the invention relates to providing a peptide consisting of at most 16 amino acids comprising one or more of the amino acid sequences set forth in SEQ ID NOS: 2-14, 46-107, 115-185 and 195-587.

In yet another embodiment, the invention relates to providing a peptide consisting of at most 20 amino acids comprising one or more of the amino acid sequences set forth in SEQ ID NOS: 46-107, 115-185 and 195-587.

In yet still another embodiment, the invention relates to providing a peptide consisting of at most 24 amino acids comprising one or more of the amino acid sequences set forth in SEQ ID NOS: 46-89, 117-124, 139-157, 163-175 and 195-587.

In yet another embodiment, the invention relates to providing a peptide consisting of at most 28 amino acids comprising one or more of the amino acid sequences set forth in SEQ ID NOS: 46-52, 61-66, 73-89, 117-124, 165-175 and 195-587.

In still another embodiment, the invention relates to providing a peptide consisting of at most 32 amino acids comprising one or more of the amino acid sequences set forth in SEQ ID NOS: 46-52, 61-66, 73-89, 117-124, 165-175 and 195-587.

In still further another embodiment, the invention relates to providing a peptide consisting of at most 36 amino acids comprising one or more of the amino acid sequences set forth in SEQ ID NO: 46-52, 61-66, 73-89, 117- 124, 165-175 and 195-587.

In yet another embodiment, the invention relates to providing a peptide consisting of at most 40 amino acids comprising one or more of the amino acid sequences set forth in SEQ ID NO: 46-52, 61-66, 73-89, 117- 124, 165-175 and 195-587.

In yet still another embodiment, the invention relates to providing a peptide consisting of at most 44 amino acids comprising one or more of the amino acid sequences set forth in SEQ ID NO: 46-52, 61-66, 73-89, 117- 124, 165-175 and 195-587.

Furthermore, in yet another embodiment, the invention relates to providing a peptide consisting of at least 48 amino acids and at most 224 amino acids comprising one or more of the amino acid sequences set forth in SEQ ID NOS: 2-587.

According to amino acid sequences of the above peptides may be derived from the amino acid sequence of polypeptides selected from the group comprising hHBP (SEQ ID NO: 1), pHBP (SEQ ID NO: 588), or human neutrophil elastase (SEQ ID NO: 589).

In one embodiment the peptide comprises at least two of the sequences set forth above. In such embodiment it is preferred that the two or more sequences constitute a continuous sequence derived from another sequence, such as a continuous sequence derived from hHBP, or pHBP, or human neutrophil elastase.

In another embodiment the peptide comprises at least two of the sequences set forth above. In such embodiment it is preferred that the two or more sequences are randomly selected to constitute a continuous sequence derived from another sequence, such as a random sequence derived from hHBP, or pHBP, or human neutrophil elastase.

In the present context by the term "derived from" is meant that one amino acid sequence, such as for example a peptide amino acid sequence, is representing a fragment, or is comprising a fragment of another amino acid sequence, such as for example the amino acid sequence of a larger polypeptide: thus, the peptide amino acid sequence is derived from (originates from) the amino acid sequence of the larger polypeptide.

In an additional embodiment the present invention relates to providing a peptide consisting of at least 24 amino acids and at most 224 amino acids comprising one or more of the amino acid sequences set forth in SEQ ID NOS: 233-253.

In another additional embodiment the present invention relates to providing a peptide consisting of at least 24 amino acids and at most 224 amino acids comprising one or more of the amino acid sequences set forth in SEQ ID NOS: 286-346.

In a preferred embodiment the present invention relates to providing a peptide having the sequence KQGRPFCAGALVHPRFVLTAASCFR set forth in SEQ ID NO: 593, or fragments of said sequence, or variants of said sequence, or fragments of said variants.

In another preferred embodiment the present invention relates to providing a peptide having the sequence NQGRHFCGGALIHARFVMTAASCFQ set forth in SEQ ID NO: 594, or fragments of said sequence, or variants of said sequence, or fragments of said variants.

By the term of "fragment" in the present context is meant that a peptide of the invention is represented by a shorter amino acid sequence which is identical to any of the amino acid sequences which the peptide comprises.

By the term "variant" in the present context is meant that a peptide of the invention is represented by an amino acid sequence which has at least 40% identity with the amino acid sequence of the peptide, more preferably at least 50%, even more preferably at least 60%, even more preferably at least 70%, even more preferably at

least 80%, even more preferably at least 90%, even more preferably at least 95%, and most preferably at least 97%.

The amino acid sequence of a variant of a peptide may differ from the amino acid sequence of the peptide by an insertion or deletion of one or more amino acid residues and/or the substitution of one or more amino acid residues by different amino acid residues. Preferably, amino acid changes are of a minor nature, that is, conservative amino acid substitutions; small deletions, typically of one to about 10 amino acids; small amino- or carboxyl-terminal extensions; small linker sequences of about 3-15 residues; or a small extension that may facilitates purification by changing net charge or another function, such as a polyhistidine tract, an antigenic epitope or a binding domain.

Examples of conservative substitutions are within the group of basic amino acids (such as arginine, lysine and histidine), acidic amino acids (such as glutamic acid and aspartic acid), polar amino acids (such as glutamine and asparagine), hydrophobic amino acids (such as leucine, isoleucine and valine), aromatic amino acids (such as phenylalanine, tryptophan and tyrosine) and small amino acids (such as glycine, alanine, serine, threonine and methionine). Amino acid substitutions, which do not generally alter the specific activity, are known in the art and are described, e.g., by H. Neurath and R.L. Hill, 1979, in, The Proteins, Academic Press, New York. The most commonly occurring exchanges are: Ala/Ser, Val/Ile, Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Tyr/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu, Asp/Gly as well as these in reverse.

It is an additional aspect of the present invention to provide functional fragments or variants of the peptides.

By the term "functional" in relation to a peptide fragment or peptide variant in the present context is meant that the peptide fragment or peptide variant is capable to demonstrate one or more of the biological activities described below.

In a preferred embodiment the invention relates to providing functional fragments, variants or fragments of said variants of a peptide having the sequence KQGRPFCAGALVHPRFVLTAASCFR (SEQ ID NO: 593).

In another preferred embodiment the invention relates to providing functional fragments, variants or fragments of said variants of a peptide having the sequence NQGRHFCGGALIHARFVMTAASCFQ (SEQ ID NO: 594).

It is an object of the invention to provide a peptide, wherein said peptide comprises one or more amino acid sequences set forth in SEQ ID NOS: 233-253 having the motif cys-X_{15}-cys, wherein X_{15} represents an amino acid sequence of 15 amino acids.

In a further embodiment said peptide comprises one or more amino acid sequences set forth in SEQ ID NOS: 233-253 and the amino acid sequence set forth in SEQ ID NO: 34.

In yet a further embodiment the present peptide comprises one or more amino acid sequences set forth in SEQ ID NOS: 233-253 and the amino acid sequence set forth in SEQ ID NO: 21.

In another aspect the peptide comprises one or more amino acid sequences set forth in SEQ ID NOS: 233-253 and the amino acid sequence set forth in SEQ ID NO: 34 and the amino acid sequence set forth in SEQ ID NO: 21.

Furthermore, it is within the scope of the invention to provide a peptide, which

i) comprises a sequence derived from any of the amino acid sequences set forth in SEQ ID NO: 1, SEQ ID NO: 588, or SEQ ID NO: 589, and
ii) is capable of bactericidal activity, and/or
iii) is an attractant for monocytes.

In one embodiment said peptide is consisting of at most 8 amino acids, whereof at least 5 and at most 6 amino acids are basic amino acids selected from the group comprising lysine, arginine and histidine.

In another embodiment the peptide is consisting of at most 12 amino acids, whereof at least 6 and at most 9 amino acids are basic amino acids selected from the group comprising lysine, arginine and histidine.

In still another embodiment the peptide is consisting of most 16 amino acids, whereof at least 8 and most 12 amino acids are basic amino acids selected from the group comprising lysine, arginine and histidine.

In still further another embodiment the peptide is consisting of at the most 20 amino acids, whereof at least 10 and at most 15 amino acids are basic amino acids selected from the group comprising lysine, arginine and histidine.

In still yet another embodiment the peptide is consisting of at most 24 amino acids, whereof at least 12 and at most 18 amino acids are basic amino acids selected from the group comprising lysine, arginine and histidine.

In yet another embodiment the peptide is consisting of at most 28 amino acids, whereof at least 14 and at most 21 amino acids are basic amino acids selected from the group comprising lysine, arginine and histidine.

In yet further another embodiment the peptide is consisting of at most 32 amino acids, whereof at least 16 and at most 24 amino acids are basic amino acids selected from the group comprising lysine, arginine and histidine.

In yet still further another embodiment the peptide is consisting of at most 36 amino acids, whereof at least 18 and at most 27 amino acids are basic amino acids selected from the group comprising lysine, arginine and histidine.

In a further another embodiment the peptide is consisting of at most 40 amino acids, whereof at least 20 and at most 30 amino acids are basic amino acids selected from the group comprising lysine, arginine and histidine.

Moreover, the invention also provides a peptide, which

i) comprises a sequence derived from any of the amino acid sequences set forth in SEQ ID NO: 1, SEQ ID NO: 588, or SEQ ID NO: 589, and

ii) is capable of preventing cell apoptosis.

In one embodiment said peptide is consisting of at most 8 amino acids, whereof at least 4 and at most 6 amino acids are acidic amino acids selected from the group comprising aspartic acid and glutamic acid.

In another embodiment the peptide in consisting of at most 12 amino acids, whereof at least 6 and at most 10 amino acids are acidic amino acids selected from the group comprising aspartic acid and glutamic acid.

In still another embodiment the peptide is consisting of at most 16 amino acids, whereof at least 8 and at most 12 amino acids are acidic amino acids selected from the group comprising aspartic acid and glutamic acid.

In yet another embodiment the peptide is consisting of at most 20 amino acids, whereof at least 10 and at most 12 amino acids are acidic amino acids selected from the group comprising aspartic acid and glutamic acid.

In still yet another embodiment the peptide is consisting of at most 24 amino acids, whereof at least 12 and at most 18 amino acids are acidic amino acids selected from the group comprising aspartic acid and glutamic acid.

In yet further another embodiment the peptide is consisting of at most 28 amino acids, whereof at least 14 and at most 21 amino acids are acidic amino acids selected from the group comprising aspartic acid and glutamic acid.

In yet still further another embodiment of at most 32 amino acids, whereof at least 16 and at most 24 amino acids are acidic amino acids selected from the group comprising aspartic acid and glutamic acid.

Furthermore, in yet still further another embodiment the peptide is consisting of at most 36 amino acids, whereof at least 18 and at most 27 amino acids are acidic amino acids selected from the group comprising aspartic acid and glutamic acid.

In a further another embodiment the peptide is consisting of at most 40 amino acids, whereof at least 20 and at most 30 amino acids are acidic amino acids selected from the group comprising aspartic acid and glutamic acid.

Screening assays

According to the invention recombinant or synthetically produced peptides are further screened for their biological activity.

In the present content by "biological activity of a peptide" is meant that a peptide is able to demonstrate at least one of the following biological activities: (1) heparin binding, (2) lipopolysaccaride (LPS) binding; (3) activating of protein kinase C; (4) stimulating thrombospondin secretion from monocytes; (5) stimulating/inhibiting the production of IL-1, IL-6, IL-8, GCSF, GM-CSF, M-CSF, TNF-α, MCP-1, group Tissue factor, IL-2R-α; (6) bactericidal; (7) chemotactic for monocytes; (8) anti-apoptotic, (9) stimulating/inhibiting the vascular permeability; (10) stimulating/inhibiting the expression of adhesion molecules PECAM or ICAM1 by endothelial cells, (11) stimulating/inhibiting the production of bradykinin, (12) increase the phagocytic potential, (13) up-regulate class-II MHC.

In a preferred embodiment the peptide is able to demonstrate at least two of the above activities, more preferably at least tree of the above activities, even more preferably at least four of the above activities (1-11), yet even more preferably at least five of the above activities, even more preferably at least six of the above activities, even more preferably at least seven of the above activities, even more preferably at least eight of the above activities, even more preferably at least nine of the above activities, even more preferably at least ten of the above activities and most preferably the peptide is able to demonstrate at least all of the above activities.

Methods for evaluating of the above listed biological activities of peptides according to the invention are well known in art.

According to the invention there are a number of available assays for evaluating the biological activity of the present peptide.

One of such assays for the evaluation of chemotactic activity of the peptides may for example be the method of Cates et al. (in Leukocyte chemotaxis, p 67. Gallin and

Quie eds, Raven Press, NY, 1978), or of Keire et al. (J. Biol. Chem. 2001, 276: 48847-53).

In another embodiment the lipopolysaccharide-binding activity of the peptides may be examined by a method described by Linde et al (Biotechniques 2000, 28:218-20).

To evaluate the bactericidal activity of the present peptides, the assay described by Shafer et al. (Infect. Immun. 1986, 53:651-55) may be used.

In one aspect measuring cell apoptopsis in the presence of the present peptides may be done according to Linde et al. (Anal. Biochem. 2000, 280:186-8).

It is possible to perform an evaluation of the heparin binding capacity of the peptides by conventional chromatography on a commercially available heparin-affinity column.

The protein kinase C activation by the peptides may be done according to Pereira et al., 1996 (J. Leukoc. Biol. 60:415-22).

The changes in expression of different polypeptides, such as for example IL-1, IL-6, IL-8, TNF-α, thrombospondin, PECAM or ICAM in the presence of the peptides according to the invention may, for example, be evaluated either by reverse phase transcriptase, immunoassay, immunoblotting, or immunostaining of the treated cells grown in culture.

The vascular permeability may be determined by using the assay as described by Gautam et al. in 1998 (Br J Pharmacol 1998 Nov;125(5):1109-14)

Medicament

It is an important objective of the present invention to use the peptides, functionally active fragments or variants of said peptides for the manufacture of a medicament for prevention and/or treatment of Gram positive and/or Gram negative infections, sepsis, severe sepsis, septic shock and/or disseminated intravascular coagulation, and/or for modulation of inflammatory response, and/or prevention of cell apoptosis.

In one embodiment the invention relates to the manufacture of a medicament which is capable of being used for prevention and/or treatment of Gram positive bacterial infection caused by Bacillaceae, Micrococcaceae, Mycobacteriaceae, Peptococcaceae and/or a Gram negative bacterial infection caused by Acetobacteriaceae, Alcaligenaceae, Bacteroidaceae, Chromatiaceae, Enterobacteriaceae, Legionellaceae, Neisseriaceae, Nitrobacteriaceae, Pseudomonadaceae, Rhizobiaceae, Rickettsiaceae, Spirochaetaceae, Vibrionaceae, Brucella, Chromobacterium.

In a preferred embodiment for prevention and/or treatment the infection by *Neisserie meningitidis* (meningococcus) and/or *Pneumococcus pneumonae* (pneumococcus).

In another embodiment the invention relates to the manufacture of a medicament which is capable of being used for prevention and/or treatment of sepsis, severe sepsis, septic shock and disseminated intravascular coagulation.

It is an important objective of the invention to use the peptides for the manufacture of a medicament for stimulation of an inflammatory response, in a preferred embodiment, the inflammatory response to bacterial infection.

Another important objective of the invention is to use the peptides for the manufacture of a medicament for inhibition of an inflammatory response. Examples of inflammatory responses, which may be harmful for an individual and therefore are advantageously being suppressed include but are not limited by conditions associated with extensive trauma, or chronic inflammation, such as for example type IV delayed hypersensitivity, associated for example with infection by *Tubercle bacilli*, or systematic inflammatory response syndrome, or multiple organ failure, or rheumatoid arthritis.

Additional important objective of the invention is to use an antibody directed against directed against an epitope comprising one or more of the sequences set forth in SEQ ID NO: 2-11, 40-232, 254-394, 421-587, said antibody is capable of stimulating the inflammatory response associated with one or more biological activities of hHBP or a hHBP fragment comprising amino acids 20-44 according to the sequence identified as SEQ ID NO: 1 for manufacture of a medicament for stimulation of an in-

flammatory response, in a preferred embodiment, the inflammatory response to bacterial infection.

Another additional objective of the present invention to use an antibody directed against an epitope comprising one or more of the sequences set forth in SEQ ID NO: SEQ ID NOS: 12-39, 233-253, 395-421, said antibody is capable of inhibiting the inflammatory response associated with one or more biological activities of hHBP or a hHBP fragment comprising amino acids 20-44 according to the sequence identified as SEQ ID NO: 1, for the manufacture of a medicament for pathological conditions wherein inhibition of inflammatory response is required. Examples of inflammatory responses, when the a medicament comprising the inhibitory antibody may be used include but are not limited by conditions associated with extensive trauma, or chronic inflammation, such as for example type IV delayed hypersensitivity, associated for example with infection by *Tubercle bacilli*, or systematic inflammatory response syndrome, or multiple organ failure, or rheumatoid arthritis.

In an additional embodiment of the invention to use the peptides capable of anti-apoptotic activity are used for the manufacture of a medicament for the treatment of a disease, pathological conditions whereof are associated with massive cell loss due to apoptosis. Examples of such a disease include but not limited by degenerative diseases the central and peripheral nervous system, such as postoperative nerve damage, traumatic nerve damage, e.g. resulting from spinal cord injury, impaired myelination of nerve fibers, postischaemic damage, e.g. resulting from a stroke, multiinfarct dementia, multiple sclerosis, nerve degeneration associated with diabetes mellitus, neuro-muscular degeneration, schizophrenia, Alzheimer's disease, Parkinson's disease, or Huntington's disease, degenerative conditions of the gonads, of the pancreas, such as diabetes mellitus type I and II, of the kidney, such as nephrosis, or cancer.

By the term "apoptosis" in the present content is meant a programmed cell death due to activation an internal death program.

In the pharmaceutical composition of a medicament of the invention, the peptides and antibodies may be formulated by any of the established methods of formulating pharmaceutical compositions, e.g. as described in Remington's Pharmaceutical Sci-

ences, 1985. The composition may typically be in a form suited for local or systemic injection or infusion and may, as such, be formulated with sterile water or an isotonic saline or glucose solution. The compositions may be sterilised by conventional sterilisation techniques, which are well known in the art. The resulting aqueous solutions may be packaged for use or filtered under aseptic conditions and lyophilised, the lyophilised preparation being combined with the sterile aqueous solution prior to administration. The composition may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as buffering agents, tonicity adjusting agents and the like, for instance sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, etc. The concentration of peptides may vary widely, i.e. from less than about 0.5%, such as from 1%, to as much as 15-20% by weight. A unit dosage of the composition may typically contain from about 10 mg to about 1 g of a peptide.

The peptides and antibodies may be administered topically or by injection. Dosages will be prescribed by the physician according to the particular condition and the particular individual to be treated. Dosages and frequency is carefully adapted and adjusted according to parameters determined by the physician in charge. A preferred administration route may be e.g. subcutaneous injections. Subcutaneous, intravenous, intramuscular, intratracheal, intravesical, intratechal or intraperitoneal injections of HBP peptides and anti-HBP antibodies may be given per 24 hours in the range of from 0.1-100 mg, especially 0.1-20 mg, in particular 0.1-10 mg per kg body weight. The dose may be given 1-4 times per 24 hours or administered continuously through a catheter.

Compositions of a medicament used in the present invention comprising bioactive peptides of HBP, HBP homologous peptides or anti-HBP antibodies described below may additionally be supplemented by antibiotics, wherein said antibiotics are routinely prescribed antibiotics by the physician according to the particular condition and the particular individual to be treated. In a preferred embodiment the supplemented antibiotics are selected from but not limited by the group of beta-lactam antibiotics, comprising penicillins and cephalosporins. A medicament comprising a peptide of HBP or a fragment of a HBP homologous peptide or an anti-HBP antobody may still additionally be supplemented by an pro-inflammatory drug, or an anti-inflammatory drug, wherein said drugs are prescribed by the physician according to

the particular condition and the particular individual to be treated. The supplementary pro-inflammatory drugs may for example be selected from the group comprising CSF (colony stimulating factor) drugs. The supplementary anti-inflammatory drugs may for example be selected from the group comprising antibiotics, steroids, cytostatics, or antiviral drugs.

HBP receptors and binding sites

According to the invention information concerning potential HBP receptors and binding sites is aiding the selection of the present peptides. There has been no identification of a HBP receptor, but receptor-like structures or binding sites of HBP have been identified. HBP is a dipole separated by a hydrophobic cleft and it is therefore capable to interact with both positively and negatively charge surfaces and molecules and with hydrophobic molecules and epitomes. The charged surface areas (the epitomes) of HBP are important for several of its functions. Without being bound by theory some of such functions are described below:

It has been demonstrated that HBP's positively charged epitomes bind to negatively charged macromolecules such as the heparan sulphate and chondroitin sulphate side chains of the proteoglycans (Olofsson, AM. et al. 1999), which are present at the surface of nearly every adherent mammalian cells. Proteoglycans are proteins with long carbohydrate chains of the glucosaminoglycans (GAG) type attached. They have recently been recognized as an important part of the signaling mechanism between cells. The proteoglycans are today recognized as co-receptors that can influence how e.g. the growth factor interacts with its receptor. Co-receptors affect which signal molecules bind to the receptor, how strong the interaction is or how far the signal spreads. Co-receptors regulate such decisions as when the cell divides, what type of proteins it manufactures and even if it should die. HBP has been shown to bind to the carbohydrate part (e.g. heparan sulphate) of the syndecan family of proteoglycans, which play an important role in internalization of proteins. The binding of HBP to such proteoglycans lead to uptake of HBP into endothelial cells (Olofsson, AM et al.,1999) and probably other cell types as well. Heparan sulphate and similar highly charged negative molecules of the glucosaminoglycan type may therefore serve as binding sites for HBP, mediating many of its diverse regulatory functions. In this context it is should be noted that heparan sulphate and similar glucosaminoglycans are not just simple negatively charged mole-

cules mediating a non-specific ionic interaction. In contrast e.g. the heparan sulphate are synthesized such that very diverse and subtle variations in the structure are achieved. Accordingly, the synthesized heparan sulphate molecules may fit only very specific positively charged epitopes, such as the ones found on the surface of HBP. The heparan sulphates and similar proteoglycans with GAG side chains may therefore be seen as a proper receptor or co-receptor for HBP.

HBP may also exploit its dipolar nature by activating the contact phase system. The contact phase system consists of HMWK and three other proteins which are closely bound together on the cell surface. HMWK is a large protein consisting of 6 domains, of which one (domain 4) contains the Bradykinin sequence. An electrostatic binding from a positively charged histidin-rich area in domain 5 of HMWK to negatively charged heparan sulphate (Renne, T. et al., 2000) and chondroitin sulphate (Renne, T. et al., 2001) proteoglycans contribute significantly to the binding of HMWK to cell surfaces. The activation of the contact phase system requires that the individual components (HMWK, fXII and pre-kallikrein) are brought in close contact to each other and probably also that certain conformational changes are induced. Heparin-binding protein (HBP) has been shown to play a pivotal role in activating the contact phase system (Gautam, N. 2001), and to be capable of highly effectively displacing HMWK from GAG in an in vitro model (Renne, T. 1999). This occurs most likely by formation of two electrostatic bindings, one between the negatively charged GAG on the cell surface and HBP's strongly positively charged surface area, and another between the positively charged domain 5 of HMWK and HBP's negatively charged surface area.

Further, in addition to the above-mentioned highly charged binding sites HBP also carries other putative binding sites, such as binding sites for the Lipid A part in LPS and for interaction with and activation of Protein Kinase C (PKC), see Iversen 1997 for a review.

Monofunctional HBP peptides

The peptides according to the invention having agonistic or antagonistic properties to the putative binding sites for HBP are of considerable pharmaceutical interest as drug candidates for the prevention and/or the treatment of infections, local and sys-

temic inflammatory disorders, asthma, systemic inflammatory response syndrome (SIRS), degenerative diseases (Alzheimer's disease), pain and other serious diseases and disorders (see table 1 in the Experimental section).

As outlined above HBP is by nature designed for initiation and regulation of local inflammatory defense to invading bacteria. In the situation of a local infection intact HBP is an ideal molecule for initiating, coordinating and regulating all the many different protecting mechanisms against the invading bacteria. In such situation a virulent inflammatory defense as initiated by HBP is fully appropriate and is probably needed to ensure survival of the individual. In agreement with this presumed beneficial effect of HBP, intact HBP has in animal studies been shown to be useful in prevention and treatment of severe life threatening infections and sepsis. However, in a therapeutic situation (by therapeutic is here to be understood both preventive and proper therapeutic interventions) HBP is administered differently from the natural way and therefore not all of its multiple effects may be needed or desirable.
Peptides with only one or some of the intact HBP molecule functions may therefore have significant advantages compared to the intact HBP for the treatment of specific conditions because they may be more specific, have different threshold for activation by a given process or be more powerful (displaying higher maximal efficacy).

Further, it is within the scope of the invention to provide peptides having a single function, i.e. mono-functional peptides, inhibiting specific HBP mediated processes. Below is a description of therapeutic applications, wherein the present peptides may be employed.

In the treatment of severe life threatening infections with HBP the monocyte activating and stimulating function may be the most important. To treat a lung infection for instance, HBP will most likely have to be administered systemically (e.g. as a subcutaneous injection or infusion). When administered at a site distant from the infection the ability of intact HBP to induce capillary leakage may not be advantageous, since this could lead to accumulation of neutrophils and edema formation at the administration site. While such potential side effect may be fully acceptable considering the ability of HBP to prevent or treat life-threatening infections, the use of a mono-functional HBP peptide according to the invention is to be preferred. The

mono-functional peptide may have an increased ability to activate monocytes and a decreased ability to induce capillary leakage.

Monocytes play a significant role not only in eliminating bacteria but also in eliminating certain cancer cells. A mono-functional peptide with increased efficacy for stimulating the cytotoxic ability of the monocytes and macrophages would be highly desirable.

Many degenerative diseases (e.g. Alzheimer's disease) are characterized by an increased program cell death – i.e. and increased apoptosis. This means, that the cells die faster. Agents preventing or delaying apoptosis could conceivably be of use for slowing down the development of such degenerative diseases. A mono-functional peptide of the invention having a high anti-apoptosis efficacy, but no inflammatory potential may be a potential candidate for intervention in degenerative diseases.

Further, while the inflammatory defense initiated and regulated by intact HBP is needed to insure elimination of local bacterial infections it is not ideally designed to combat systemic infections, which spread to the whole body. In such circumstances, the inflammatory response may lead to damages to the organs and even to death of the organism if not controlled or stopped in time. Furthermore, the organism may react with an inflammatory response in situations where there is no infection. In such situations the inflammatory response is not only needless it is also highly damaging to the body. As an example, patients exposed to a trauma e.g. a car accident may develop a systemic inflammatory response caused by the extensive tissue damage. This may lead to hypotension, activation of the coagulation system, formation of clots and subsequent bleeding due to increased fibrinolysis, respiratory distress and failure of vital organs, such as the liver, kidney and the heart. The mechanisms leading to such Systemic Inflammatory Response Syndrome (SIRS) and Multiple Organ Failure (MOF) have not been fully elucidated. Without being bound by theory the interaction and contribution of HBP to the activation of the contact phase system probably plays a significant role.

According to the invention it is of significant interest to provide antagonist to HBP to use in the prevention and/or the treatment of such serious disorders. While antibod-

ies to HBP may be highly useful in several clinical situations characterized by increased activation of the contact phase system and increased Bradykinin release, small peptide HBP antagonists may have several advantages. They may be used in a wider range of diseases and disorders due to their smaller size and presumed better tissue penetration.

In another aspect of the present invention a more effective means of preventing bradykinin mediated disease processes is provided. As mentioned above anti-HBP antibodies are obvious candidates, but small peptide HBP antagonists of the invention may have significant advantages. Bradykinin plays a role in the development of SIRS amongst other diseases. Bradykinin exerts its effect by interaction with specific receptors. Numerous bradykinin antagonists have been synthesized in the search for new drugs, which can prevent the action of bradykinin in conditions, such as circulatory and endotoxic shock, rhinitis and other allergic conditions, chronic inflammatory diseases such as rheumatoid arthritis, and colitis ulcerosa and brain edema. Although some of them have shown to be of some clinical use the effect has in general been less than expected considering the central mediator role of bradykinin. Without being bound by theory one reason might be that an antagonist only partly blocks the effect of bradykinin, but probably more likely that the antagonist in a therapeutic situation is often given after a significant amount of bradykinin has been released and has exerted its effects on the receptors.

In a further aspects of the present invention mono-functional, non-toxic agonists which bind LPS or other endotoxins, such as PGN, LTA or other cell wall components from bacteria with the same or higher affinity as Polymyxin B would have significant therapeutic potential for the treatment of sepsis. Endotoxins from both Gram negative (LPS) and Gram positive (PGN, LTA) bacteria play a significant role in the development of septic shock. It has recently been shown that removal of endotoxin (LPS) from the blood of septic patient by passage of the patient's blood through a Polymyxin B – column significantly reduces mortality. Polymyxin B binds LPS. Polymyxin B has certain structural similarities with HBP, which also binds LPS.

Production

The peptides of the present invention may be prepared by conventional synthetic methods, recombinant DNA technologies, enzymatic cleavage of full-length proteins which the peptide sequences are derived from, or a combination of said methods.

Synthetic preparation

The methods for synthetic production of peptides are well known in art. Detailed descriptions as well as practical advice for producing synthetic peptides may be found in Synthetic Peptides: A User's Guide (Advances in Molecular Biology), Grant G. A. ed., Oxford University Press, 2002, or in: Pharmaceutical Formulation: Development of Peptides and Proteins, Frokjaer and Hovgaard eds., Taylor and Francis, 1999.

Peptides may for example be synthesised by using Fmoc chemistry and with Acm-protected cysteins. After purification by reversed phase HPLC, peptides may be further processed to obtain for example cyclic or C- or N-terminal modified isoforms. The methods for cyclization and terminal modification are well-known in the art and described in detail in the above cited manuals.

The DNA sequence encoding a peptide or full-length protein of the invention may be prepared synthetically by established standard methods, e.g. the phosphoamidine method described by Beaucage and Caruthers, 1981, Tetrahedron Lett. 22:1859-1869, or the method described by Matthes et al., 1984, EMBO J. 3:801-805. According to the phosphoamidine method, oligonucleotides are synthesized, e.g. in an automatic DNA synthesizer, purified, annealed, ligated and cloned in suitable vectors.

Recombinant preparation

The peptides of the invention may also be produced by use of recombinant DNA technologies. The DNA sequence encoding a peptide may be prepared by fragmentation of the DNA sequences encoding a full-length protein, which the peptide is derived from, using DNAase I according to a standard protocol (Sambrook et al., Molecular cloning: A Laboratory manual. 2 rd ed., CSHL Press. Cold Spring Harbor, NY, 1989). The present invention relates to the full-length protein being selected from the group of proteins comprising human HBP (SEQ ID NO:1), porcine HBP (SEQ ID

NO: 588) and human neutrophil elastase (SEQ ID NO: 589), said proteins being encoded by the DNA sequences set forth in SEQ ID NO: 590, SEQ ID NO: 591 and SEQ ID NO: 592, correspondingly. The DNA encoding the full-length proteins may alternatively be fragmented using specific restriction endonucleases. The fragments of DNA are further purified using standard procedures described in Sambrook et al., Molecular cloning: A Laboratory manual. 2 rd ed., CSHL Press, Cold Spring Harbor, NY, 1989.

The DNA sequence encoding a full-length protein may also be of genomic or cDNA origin, for instance obtained by preparing a genomic or cDNA library and screening for DNA sequences coding for all or part of the full-length protein by hybridization using synthetic oligonucleotide probes in accordance with standard techniques (cf. Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor, 1989). The DNA sequence may also be prepared by polymerase chain reaction using specific primers, for instance as described in US 4,683,202 or Saiki et al., 1988, Science 239:487-491.

The DNA sequence is then inserted into a recombinant expression vector, which may be any vector, which may conveniently be subjected to recombinant DNA procedures. The choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector that exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the DNA sequence encoding a peptide or a full-length protein should be operably connected to a suitable promoter sequence. The promoter may be any DNA sequence, which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. Examples of suitable promoters for directing the transcription of the coding DNA sequence in mammalian cells are the SV 40 promoter (Subramani et al., 1981, Mol. Cell Biol. 1:854-864), the MT-1 (metallothionein gene) promoter (Palmiter et al., 1983, Science 222: 809-814) or the adenovirus 2 major late promoter. A suitable promoter for use in insect cells is the polyhedrin promoter (Vasu-

vedan et al., 1992, FEBS Lett. 311:7-11). Suitable promoters for use in yeast host cells include promoters from yeast glycolytic genes (Hitzeman et al., 1980, J. Biol. Chem. 255:12073-12080; Alber and Kawasaki, 1982, J. Mol. Appl. Gen. 1: 419-434) or alcohol dehydrogenase genes (Young et al., 1982, In Genetic Engineering of Microorganisms for Chemicals, Hollaender et al, eds., Plenum Press, New York), or the TPI1 (US 4,599,311) or ADH2-4c (Russell et al., 1983, Nature 304:652-654) promoters. Suitable promoters for use in filamentous fungus host cells are, for instance, the ADH3 promoter (McKnight et al., 1985, EMBO J. 4:2093-2099) or the tpiA promoter.

The coding DNA sequence may also be operably connected to a suitable terminator, such as the human growth hormone terminator (Palmiter et al., op. cit.) or (for fungal hosts) the TPI1 (Alber and Kawasaki, op. cit.) or ADH3 (McKnight et al., op. cit.) promoters. The vector may further comprise elements such as polyadenylation signals (e.g. from SV 40 or the adenovirus 5 Elb region), transcriptional enhancer sequences (e.g. the SV 40 enhancer) and translational enhancer sequences (e.g. the ones encoding adenovirus VA RNAs).

The recombinant expression vector may further comprise a DNA sequence enabling the vector to replicate in the host cell in question. An example of such a sequence (when the host cell is a mammalian cell) is the SV 40 origin of replication. The vector may also comprise a selectable marker, e.g. a gene the product of which complements a defect in the host cell, such as the gene coding for dihydrofolate reductase (DHFR) or one which confers resistance to a drug, e.g. neomycin, hydromycin or methotrexate.

The procedures used to ligate the DNA sequences coding the peptides or full-length proteins, the promoter and the terminator, respectively, and to insert them into suitable vectors containing the information necessary for replication, are well known to persons skilled in the art (cf., for instance, Sambrook et al., op.cit.).

To obtain recombinant peptides of the invention the coding DNA sequences may be usefully fused with a second peptide coding sequence and a protease cleavage site coding sequence, giving a DNA construct encoding the fusion protein, wherein the protease cleavage site coding sequence positioned between the HBP fragment and second peptide coding DNA, inserted into a recombinant expression vector, and

expressed in recombinant host cells. In one embodiment, said second peptide selected from, but not limited by the group comprising glutathion-S-reductase, calf thymosin, bacterial thioredoxin or human ubiquitin natural or synthetic variants, or peptides thereof. In another embodiment, a peptide sequence comprising a protease cleavage site may be the Factor Xa, with the amino acid sequence *IEGR*, enterokinase, with the amino acid sequence *DDDDK*, thrombin, with the amino acid sequence *LVPRGS*, or *Acharombacter lyticus*, with the amino acid sequence *XKX*, cleavage site.

Host cell

The host cell into which the expression vector is introduced may be any cell which is capable of expression of the peptides or full-length proteins, and is preferably a eukaryotic cell, such as invertebrate (insect) cells or vertebrate cells, e.g. *Xenopus laevis* oocytes or mammalian cells, in particular insect and mammalian cells. Examples of suitable mammalian cell lines are the HEK293 (ATCC CRL-1573), COS (ATCC CRL-1650), BHK (ATCC CRL-1632, ATCC CCL-10) or CHO (ATCC CCL-61) cell lines. Methods of transfecting mammalian cells and expressing DNA sequences introduced in the cells are described in e.g. Kaufman and Sharp, J. Mol. Biol. 159, 1982, pp. 601-621; Southern and Berg, 1982, J. Mol. Appl. Genet. 1:327-341; Loyter et al., 1982, Proc. Natl. Acad. Sci. USA 79: 422-426; Wigler et al., 1978, Cell 14:725; Corsaro and Pearson, 1981, in Somatic Cell Genetics 7, p. 603; Graham and van der Eb, 1973, Virol. 52:456; and Neumann et al., 1982, EMBO J. 1:841-845.

Alternatively, fungal cells (including yeast cells) may be used as host cells. Examples of suitable yeast cells include cells of *Saccharomyces spp.* or *Schizosaccharomyces spp.*, in particular strains of *Saccharomyces cerevisiae*. Examples of other fungal cells are cells of filamentous fungi, e.g. *Aspergillus spp.* or *Neurospora spp.*, in particular strains of *Aspergillus oryzae* or *Aspergillus niger*. The use of *Aspergillus spp.* for the expression of proteins is described in, e.g., EP 238 023.

Culture medium

The medium used to culture the cells may be any conventional medium suitable for growing mammalian cells, such as a serum-containing or serum-free medium containing appropriate supplements, or a suitable medium for growing insect, yeast or

fungal cells. Suitable media are available from commercial suppliers or may be prepared according to published recipes (e.g. in catalogues of the American Type Culture Collection).

The peptides or full-length proteins recombinantly produced by the cells may then be recovered from the culture medium by conventional procedures including separating the host cells from the medium by centrifugation or filtration, precipitating the proteinaceous components of the supernatant or filtrate by means of a salt, e.g. ammonium sulphate, purification by a variety of chromatographic procedures, e.g. HPLC, ion exchange chromatography, affinity chromatography, or the like.

Antibody

Another aspect of the present invention concerns the providing of an antibody directed against an epitope comprising one or more of the sequences set forth in SEQ ID NO: SEQ ID NOS: 12-39, 233-253, 395-421, said antibody is capable of inhibiting the inflammatory response associated with one or more biological activities of hHBP or a hHBP fragment comprising amino acids 20-44 according to the sequence identified as SEQ ID NO: 1, and an antibody directed against an epitope comprising one or more of the sequences set forth in SEQ ID NO: 2-11, 40-232, 254-394, 421-587, said antibody is capable of stimulating the inflammatory response associated with one or more biological activities of hHBP or a hHBP fragment comprising amino acids 20-44 according to the sequence identified as SEQ ID NO: 1.

Antibody molecules belong to a family of plasma proteins called immunoglobulins, whose basic building block, the immunoglobulin fold or domain, is used in various forms in many molecules of the immune system and other biological recognition systems. A typical immunoglobulin has four polypeptide chains, containing an antigen binding region known as a variable region and a non-varying region known as the constant region.

Native antibodies and immunoglobulins are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of

different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant domains. Each light chain has a variable domain at one end (VL) and a constant domain at its other end. The constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light and heavy chain variable domains (Clothia et al., J. Mol. Biol. 186, 651-66, 1985); Novotny and Haber, Proc. Natl. Acad. Sci. USA 82, 4592-4596 (1985).

Depending on the amino acid sequences of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are at least five (5) major classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, and several of these may be further divided into subclasses (isotypes), e.g. IgG-1, IgG-2, IgG-3 and IgG-4; IgA-1 and IgA-2. The heavy chains constant domains that correspond to the different classes of immunoglobulins are called alpha (α), delta (δ), epsilon (ε), gamma (γ) and mu (μ), respectively. The light chains of antibodies can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino sequences of their constant domain. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The term "variable" in the context of variable domain of antibodies, refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies. The variable domains are for binding and determine the specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed through the variable domains of antibodies. It is concentrated in three segments called complementarity determining regions (CDRs) also known as hypervariable regions both in the light chain and the heavy chain variable domains.

The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely a adopting a β-sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and,

with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies. The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

An antibody that is contemplated for use in the present invention thus can be in any of a variety of forms, including a whole immunoglobulin, an antibody fragment such as Fv, Fab, and similar fragments, a single chain antibody which includes the variable domain complementarity determining regions (CDR), and the like forms, all of which fall under the broad term "antibody", as used herein. The present invention contemplates the use of any specificity of an antibody, polyclonal or monoclonal, and is not limited to antibodies that recognize and immunoreact with a specific antigen. In preferred embodiments, in the context of both the therapeutic and screening methods described below, an antibody or fragment thereof is used that is immunospecific for an antigen or epitope of the invention.

The term "antibody fragment" refers to a portion of a full-length antibody, generally the antigen binding or variable region. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments. Papain digestion of antibodies produces two identical antigen binding fragments, called the Fab fragment, each with a single antigen binding site, and a residual "Fc" fragment, so-called for its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen binding fragments that are capable of cross-linking antigen, and a residual other fragment (which is termed pFc'). Additional fragments can include diabodies, linear antibodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments. As used herein, "functional fragment" with respect to antibodies, refers to Fv, F(ab) and $F(ab')_2$ fragments.

Antibody fragments may be as small as about 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, 9 amino acids, about 12 amino acids, about 15 amino acids, about 17 amino acids, about 18 amino acids, about 20 amino acids, about 25 amino acids, about 30 amino acids or more. In general, an antibody fragment of the invention can have any upper size limit so long as it is has similar or immunological properties relative to antibody that binds with specificity to an epitope formed by any of SEQ ID NO: 2-587.

Antibody fragments retain some ability to selectively bind with its antigen or receptor. Some types of antibody fragments are defined as follows:

(1) Fab is the fragment that contains a monovalent antigen-binding fragment of an antibody molecule. A Fab fragment can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain.

(2) Fab' is the fragment of an antibody molecule can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain. Two Fab' fragments are obtained per antibody molecule.

Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH1 domain including one or more cysteines from the antibody hinge region.

(3) $(Fab')_2$ is the fragment of an antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction.

(4) $F(ab')_2$ is a dimer of two Fab' fragments held together by two disulfide bonds.

Fv is the minimum antibody fragment that contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, non-covalent association (V_H -V_L dimer). It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the V_H -V_L dimer. Collectively, the six CDRs confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

(5) Single chain antibody ("SCA"), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule. Such single chain antibodies are also referred to as "single-chain Fv" or "sFv" antibody fragments. Generally, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains that enables the sFv to form the desired structure for antigen binding. For a review of sFv see Pluckthun in The

Pharmacology of Monoclonal Antibodies 113: 269-315 Rosenburg and Moore eds. Springer-Verlag, NY, 1994.

The term "diabodies" refers to a small antibody fragments with two antigen-binding sites, which fragments comprise a heavy chain variable domain (VH) connected to a light chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161, and Hollinger et al., Proc. Natl. Acad Sci. USA 90: 6444-6448 (1993).

The preparation of polyclonal antibodies is well-known to those skilled in the art. See, for example, Green et al. 1992. Production of Polyclonal Antisera, in: Immunochemical Protocols (Manson, ed.), pages 1-5 (Humana Press); Coligan, et al., Production of Polyclonal Antisera in Rabbits, Rats Mice and Hamsters, in: Current Protocols in Immunology, section 2.4.1, which are hereby incorporated by reference.

The preparation of monoclonal antibodies likewise is conventional. See, for example, Kohler & Milstein, Nature, 256:495 (1975); Coligan, et al., sections 2.5.1-2.6.7; and Harlow, et al., in: Antibodies: A Laboratory Manual, page 726 (Cold Spring Harbor Pub. (1988)), which are hereby incorporated by reference. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, e.g., Coligan, et al., sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes, et al., Purification of Immunoglobulin G (IgG). In: Methods in Molecular Biology, 1992, 10:79-104, Humana Press, NY.().

Methods of *in vitro* and *in vivo* manipulation of monoclonal antibodies are well known to those skilled in the art. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, Nature 256, 495 (1975), or may be made by recombinant methods, e.g., as described in US 4,816,567. The monoclonal antibodies for use with the present invention may also be isolated from phage anti-

body libraries using the techniques described in Clackson et al., 1991, Nature 352: 624-628, as well as in Marks et al., 1991, J Mol Biol 222: 581-597. Another method involves humanizing a monoclonal antibody by recombinant means to generate antibodies containing human specific and recognizable sequences. See, for review, Holmes, et al., 1997, J Immunol 158:2192-2201 and Vaswani, et al., 1998, Annals Allergy, Asthma & Immunol 81:105-115.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In additional to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (US 4,816,567); Morrison et al., 1984, Proc Natl Acad Sci 81, 6851-6855.

Methods of making antibody fragments are also known in the art (see for example, Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, NY, 1988, incorporated herein by reference). Antibody fragments of the present

invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, in US 4,036,945 and US 4,331,647, and references contained therein. These patents are hereby incorporated in their entireties by reference.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody. For example, Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent or the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by Whitlow, et al., 1991, In: Methods: A Companion to Methods in Enzymology, 2:97; Bird et al., 1988, Science 242:423-426; US 4,946,778; and Pack, et al., 1993, BioTechnology 11:1271-77.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") are often involved in antigen recognition and binding. CDR peptides can be obtained by cloning or constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthe-

size the variable region from RNA of antibody-producing cells. See, for example, Larrick, et al., Methods: a Companion to Methods in Enzymology, Vol. 2, page 106 (1991).

The invention contemplates human and humanized forms of non-human (e.g. murine) antibodies. Such humanized antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a nonhuman species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity.

In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, humanized antibodies will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see: Jones et al., 1986, Nature 321, 522-525; Reichmann et al., 1988, Nature 332, 323-329; Presta, 1992, Curr Op Struct Biol 2:593-596; Holmes et al., 1997, J Immunol 158:2192-2201 and Vaswani, et al., 1998, Annals Allergy, Asthma & Immunol 81:105-115.

The invention provides both polyclonal and monoclonal antibodies. The generation of antibodies may be achieved by standard methods in the art for producing polyclonal and monoclonal antibodies using a natural or recombinant HBP polypeptide or fragment thereof as an antigen. Such antibodies would be in a preferred embodiment generated using a naturally occurring or recombinantly produced HBP-related polypeptides having amino acid sequences set forth in SEQ ID

NO: 1, 588 or 589, or variants or fragments thereof, or, in a more preferred embodiment, using fragments of said polypeptides, wherein said fragments would meet at least two of the following criteria:

(i) being a natural or synthetic contiguous amino acid sequence of at least 8 amino acids;

(ii) comprising an amino acid sequence selected from SEQ ID NOS: 2-587;

(iii) derived from the sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 588, or 589 or variants of thereof;

(iv) comprising a motif of the formula X^1-X^2-Cys-X^3-X^4-X^5-X^6-X^7-X^8-X^9-X^{10}-X^{11}-X^{12}-X^{13}-X^{14}-X^{15}-X^{16}-X^{17}-Cys-X^{18}-X^{19}, wherein X is defined as described above;

(v) comprising an amino acid sequence selected from SEQ ID NOS: 593-606 ; acid residues.

In another embodiment the antibodies are produced *in vivo* by the individual to be treated, for example, by administering an immunogenic fragment according to the invention to said individual. Accordingly, the present invention further relates to a vaccine comprising an immunogenic fragment described above.

Furthermore, the invention provides monoclonal antibody F19A5B4. According to invention antibody F19A5B4 is directed against an epitope comprising one or more of the sequences set forth in SEQ ID NO: SEQ ID NOS: 12-39, 233-253, 395-421, said antibody is capable of inhibiting the inflammatory response associated with one or more biological activities of hHBP or a hHBP fragment comprising amino acids 20-44 according to the sequence identified as SEQ ID NO: 1.

The invention further provides monoclonal antibody F19A5B1. According to invention antibody F19A5B1 is directed against an epitope comprising one or more of the sequences set forth in SEQ ID NO: 2-11, 40-232, 254-394, 421-587, said antibody is capable of stimulating the inflammatory response associated with one or more biological activities of hHBP or a hHBP fragment comprising amino acids 20-44 according to the sequence identified as SEQ ID NO: 1.

An antibody provided by the invention is preferably aimed for the manufacture of a medicament for pathological conditions wherein inhibition or stimulation of inflammatory response is required.

Experimentals

Identification of and screening for active HBP peptide sequences

Any HBP peptide sequence with 4 or more amino acid residues may be able to exercise an agonistic or antagonistic function against one or more of HBP putative binding sites or receptors (for simplicity just called HBP receptors below). However, certain sequences and surface areas may be identified as more interesting than others. Several measures to identify potentially interesting sequences with agonistic or antagonistic functions to HBP receptors were taken.

Firstly, the peptide sequence of HBP and proteins closely similar to HBP within the same species I.e. Homo sapiens were investigated. HBP is structurally very similar to human neutrophil elastase (hHNE), and specific HBP functions may be found in the areas of human HBP, which are non identical to the sequences of hHNE, i.e. in the areas of the HBP molecule, which have not been conserved during evolution.

Secondly, it is hypothesized that such peptide sequences in HBP, which are conserved between species might be of particular interest.

Thirdly, it is hypothesized that sequences in other species than Homo sapiens e.g. the pig which are closely similar but not identical to the corresponding human sequence might have other abilities than the human sequence, e.g. be antagonistic instead of agonistic.

Finally, it is hypothesized that among the sequences identified as outlined above, the more interesting sequences may have to be found on the surface of the molecule. As the two more important known receptor-like surface areas (epitopes) are both highly charged it is hypothesized that sequences with high density of charged amino acids would be of particular interest.

Screening of HBP peptide sequences for biological activity

Among the many sequences identified by the above approach, only some will have desirable biological and pharmaceutical functions. The number of possible combinations (i.e. different amino acid sequences) is astronomical. As an example the theo-

retical number of different peptides consisting of 25 amino acids is $3.36 * 10^{32}$. Even though some sequences based on the above outlined considerations can be identified as more interesting than others, a high number of different peptides will have to be screened for interesting biological and pharmaceutical properties.

It is therefore important to have highly reliable high capacity assay systems to identify HBP peptides and analogues hereof with pharmaceutical potential. In this contest it should be realized that testing of peptides to be useful for preventive or therapeutic purposes in humans, should be done in a human system. Intact HBP from one species does not necessarily react identical in other species and use of an animal test system e.g. a rat system for screening of the biological functions of an HBP derived peptide to be used in humans could easily be misleading. Below examples on screening assays to be used for identification of peptides with pharmaceutical potential for prevention and treatment of infectious diseases (e.g. pneumonia), severe inflammatory disorders (e.g. SIRS) and degenerative disorders (e.g. Alzheimer's disease) are presented.

Example 1: Screening for inflammatory and anti-inflammatory potential of HBP derived peptide.

Human whole blood (WB) contains besides red cells, platelets and plasma the white blood cells including the neutrophils and monocytes. Neutrophils and monocytes have receptors for bacterial products such as LPS, PGN and LTA. The bacterial products react directly or via specific binding proteins to receptors on the monocytes thereby stimulating them to secrete and release inflammatory cytokines comprising, but not limited to IL-1, IL-6, and TNF-α. HBP has in itself no measurable effect on cytokine secretion, but significantly amplifies cytokine synthesis and secretion induced by bacterial products. In the assay type described the amplification of 160 µmol HBP per ml WB in general leads to at least three-fold amplification of the cytokine secretion.

In the example to be described LPS from the E. coli is used to stimulate the monocytes in WB (anti-coagulated by use of citrate) and the activity is measured by subsequently quantifying IL-6 in plasma separated from WB. The activity of HBP, HBP derived peptides and analogues hereof are evaluated by their ability to:

1. increase IL-6 secretion in absence of bacterial products
2. increase IL-6 secretion in presence of bacterial products
3. decrease IL-6 secretion in presence of bacterial products
4. inhibit amplification of cytokine secretion induced by intact HBP

An ideal HBP peptide agonist will display the same ability as intact HBP itself in this system, i.e. it will have no activity itself when added to WB but when added simultaneously with LPS or another bacterial product it should stimulate the IL-6 secretion with a factor of at least 3, preferably 4 or more. If the peptide itself stimulates IL-6 secretion in options or of LPS or other bacterial products, it may lead to a systemic hyper-inflammation in organism which is not desirable.

Reagents and methods

All operations must be carried out in LAF cabinet by observance of stringent aseptic techniques. All test tubes, pipette tips etc. must be pyrogen-free. Buffers must be prepared by use of sterile, pyrogen water, preferably water for injection. Use 0.1 % pyrogen-free BSA/PBS for all dilutions.

Add 20 µl of HBP derived peptide (in concentrations from 25 to 2500 µM) to 100 µl freshly drawn (less than 4 hours old) citrate whole blood from a healthy human volunteer. Add 20 µl bacterial component (LPS, LTA or PGN) in concentrations from 5 to 5000 ng/ml, preferably 50 to 500 ng/ml. Mix well and incubate for 16-18 hours in an atmosphere of 5 % carbon dioxide and at least 95 % relative humidity. At the end of the incubation add at least 5 volumes (700 µl) 0.1 % BSA/PBS. Mix well. Centrifuge 10 min. at 10.000 g. Aspirate 500 µl supernatant. Determine the level of IL-6 by a specific human immune assay for human IL-6 with sensitivity of at least 3 pg/ml, e.g. Human IL-6 Kit from RnD Systems (cat. no. D 6050).

Negative controls: 100 µl WB plus 40 µl 0.1 % BSA/PBS. Positive control: 100 µl WB plus 20 µl LPS (same concentration as used for testing the peptide) and 20 µl 0.1 % BSA/PBS.

Example 2: Screening for anti-apoptotic potential of HBP derived peptides and analogues

The screening for anti-apoptotic peptides is carried out essentially as described by Shrotri MS. et al., 2000.

Isolation of human neutrophils (PMNs)

Peripheral blood from normal volunteers was collected and PMNs were separated by density gradient using Ficoll–Hypaque (Sigma Chemical Co.). PMNs obtained were divided into 1-ml samples with 3.0 3 106 cells/ml and were treated and analyzed as per the protocols described below.

Cell Fixation and Staining Protocol for Analysis of Apoptosis

Cell pellets were obtained by centrifugation at 300*g* and fixed with 1% paraformaldehyde for 15 min at 4°C. Cells were washed twice and permeabilized with 70% ice-cold ethanol and stored at 220°C. Cells samples were washed twice and stained by the terminal dUTP nick-end labeling (TUNEL) assay using the APO-BRDU kit (Phoenix Flow Systems, San Diego, CA), following the manufacturer's instructions. Briefly, in this assay, the enzyme terminal deoxynucleotidyl transferase (TdT) catalyzes the addition of nucleotides to DNA strand breaks in the apoptotic cells, which are subsequently labeled with fluorescein isothiocyanate (FITC)-conjugated antinucleotide antibodies. The fluorescent cells are the apoptotic cells that are then identified or assessed using the flow cytometer (EPICS Elite, Beckman-Coulter, Hialeah, FL). These apoptotic cells were also visually confirmed by confocal microscopy (Meridian Instruments Inc., Okemos, MI).

Time zero group. Certain samples were fixed immediately after isolation, permeabilized, and stored at 220°C for TUNEL assay, as described earlier. These samples were designated as time zero, in which no culturing was involved.

24-h culture groups. Certain samples were re-suspended after isolation in 10% fetal bovine serum (serum-enriched group) with 25 µg of control protein (BSA) or 25 µg of HBP per ml. sample. Samples were incubated in 0% serum or RPMI (serum-deprived group) with control protein, HBP or HBP derived peptide. After 24 h culture

in a humidified CO2 incubator, cells were fixed, permeabilized, and stored at 220°C for later TUNEL assay, as described above.

Identification of anti-apoptotic peptides

Addition of 25 µg HBP per ml sample typically decreases apoptosis from about 70 % to 45 %. Peptides decreasing apoptosis to the same or greater extent when used in equimolar amounts should be considered anti-apoptotic.

Example 3: Identification of a highly potent anti-inflammatory HBP peptide

As an example on the use of the methods outlined above to predict an HBP peptide sequence it is disclosed how a highly anti-inflammatory novel HBP peptide sequence is identified.

The innate immune response is activated by pattern recognition receptors (toll like receptors) on monocytes, neutrophils and other immune cells. HBP increase the sensitivity of these pattern recognition receptors for their response to the specific pattern motifs on the cell wall of both gram negative and gram positive bacteria as well as on fungi and other infective agents. Serine proteases and more important serine proteases with mutations in the catalytic site, but with a highly conserved serine protease fold such as HBP, play a pivotal role in innate immunity. Invertebrates have only innate immune response as the specific immune system was first developed with the evolution of the bonefish. Several serine proteases with mutations in the active site has been studied in a number of invertebrates and the *Trichoplusia ni* larval has an HBP-like serine protease. The hallmark in the mutation in the active site in the HBP from man, pig and *Trichoplusia ni* is an Histidin (H) to Serine (S) mutation, a conserved Aspartic acid (D) whereas the Serine (S) mutation is random. The active site with these mutations is therefore highly likely involved in the mechanism of action of the HBP family.

Method

In the present context the term "h20-44" covers the human heparin binding protein sequence of amino acids numbers 20-44. Further, by the term "p20-44" is meant the porcine heparin binding protein sequence of amino acids numbers 20-44.

The human (h20-44) and porcine (p20-44) peptides were synthesized and tested in the screening assay described in Example 1. The following parameters were examined: (a) abiility to induce inflammation on their own (i.e. in the absence of any bacterial component), (b) ability to amplify or inhibit inflammation induced by a bacterial component, such as LPS from the Gram negative bacteria E. coli and PGN or Purified Cell Wall (PCW) from a Gram positive bacteria. As measure for immune stimulation secretion of the release of IL-6 in citrated whole blood was used. The peptides were tested in the concentrations 0.09, 0.18, 0.36 and 0.71 mg/ml blood. LPS was used at the concentration 100 ng/ml blood and PGN and PCW were used at 50 µg/ml blood.

Results

Human HBP 20-44 peptide induced a significant and dose-dependent increase in IL-6 secretion, whereas porcine HBP 20-44 displayed no significant or dose-dependent effect (see Figure 1).

In blood stimulated with 100 ng/ml LPS, human HBP 20-44 did not significantly increase the IL-6 secretion up to 0.36 mg/ml peptide. At 0.71 mg/ml, human HBP 20-44 increased IL-6 secretion significantly, but the effect of human HBP 20-44 and LPS together was slightly less (12,130 pg/ml) than the sum of the IL-6 secretion induced by LPS and human HBP 20-44 individually (14,178 pg/ml) (see Figure 2). In contrast porcine HBP 20-44 peptide significantly and dose-dependently inhibited the LPS induced IL-6 secretion.

In blood stimulated with PGN (50 µg/ml) from Staphylococcus aureus human HBP 20-44 increased IL-6 secretion in dose-dependent way (see Figure 3). In contrast porcine HBP 20-44 peptide significantly and dose-dependently inhibited the PGN induced IL-6 secretion.

In blood stimulated with PCW (50 µg/ml) from Staphylococcus aureus human HBP 20-44 increased IL-6 secretion in dose-dependent way (Figure 4). In contrast porcine HBP 20-44 peptide significantly and dose-dependently inhibited the PCW induced IL-6 secretion.

Conclusion

Human HBP 20-44 peptide surprisingly by itself significant stimulate secretion of the pro-inflammatory cytokine IL-6. In the presence of bacterial components human HBP 20-44 to some extent further increases the immune stimulation induced by the bacterial products. Human HBP 20-44 has previously been thought to act via its ability to bind to LPS and was presumed to be an LPS neutralizing agent. An LPS neutralizing agent would inhibit the immune response, but here it is shown that human HBP 20-44 is instead a powerful immune stimulating agent. In contrast the structurally very similar porcine HBP 20-44 peptide, which only deviates from its human counterpart by 7 amino acid substitutions was found to be a highly potent anti-inflammatory agent, which significantly decreases inflammation induced by bacterial components from both Gram negative and Gram positive bacteria. Porcine HBP 20-44 peptide thus holds significant potential for becoming a broadly applicable anti-inflammatory agents with indications ranging form treatment of chronic inflammatory diseases over re-perfusion injuries in myocardial and brain insults to the life-threatening systemic inflammatory response syndrome.

Example 4: Modification of the C-terminal and N-terminal amino acid residues in hHBP and pHBP.

In human blood stimulated with 250 ug/ml PGN from S. aureus, hHBP 20-44 acetylated at the N-terminal amino group and amidated at the C-terminal carboxyl group stimulated the IL-6 secretion in a dose dependant way (figure 5). In figure 5 the IL-6 response has been normalized to the effect of PGN alone. Compared to figure 2 it appears that there is a more than ten fold stimulation of the PGN mediated IL-6 production for the N- and C-terminal substituted peptide.

Example 5: Essential amino acid residues in hHBP and pHBP involved in regulation of IL-6 production.

From figures 7 and 8 it can be seen that when pHBP 20-44 was acetylated and amidated at the N- and C-terminal amino acids, respectively, the variant peptide became more inhibitory to the LPS and PGN induced IL-6 production compared to the unsubstituted peptide.

In order to identify essential amino acid residues in human and porcine HBP 20-44 involved in the effects observed (see figures 1 – 4), several variants with amino acid substitutions in defined positions were tested. From figure 6 it appears that the substitution of arginine to glutamine in position 34 of pHBP 20-44 completely abolished the inhibitory effect of the peptide in the presence of PGN.

From figures 7 and 8 it appears that when the N-terminal amino acid residue of the pHBP 20-44 was substituted with the human N-terminal amino acid residue, the variant became less inhibitory compared to the original porcine peptide in the presence of both LPS and PGN. When both the N- and C-terminal amino acid residues of hHBP 20-44 was substituted with the corresponding porcine amino acid residues, the variant became nearly non-stimulating compared to the unsubstituted human peptide in the presence of LPS and in the presence of PGN the substituted peptide became inhibitory to the IL-6 production. From figures 7 and 8 it also appears that the same pattern was observed when the C-terminal amino acid residue in hHBP 20-44 was replaced with the porcine C-terminal amino acid residue, or when C-terminal amino acid residue in pHBP 20-44 was replaced with the human C-terminal amino acid residue.

Example 6: Production of monoclonal antibodies

Production of antibodies that inhibit or enhance the IL-6 production in whole human blood in the presence or absence of h20-44 peptide or in the presence of h20-44 peptide and PGN or PCW from Stafylococcus areus.

Methods

A panel of monoclonal antibodies raised against pHBP were produced according to method of Kohler G. and Milstein C. Continous cultures of fused cells secreting antibody of predefined specificity. Nature 1975;256:495-497. The antibodies were purified using ImmunoPure^{R} IgG (Protein A) Purification Kit (PIERCE) and tested in the whole blood assay as described in example 1.

Results

Table 1

Blood	F19A5B1	F19A5B4	PHBP	0.1%BSA	PCW	IL-6

microliter	micro-gram/ml	micro-gram/ml	microgra m/ml	microliter	micro-gram/ml	pg/ml
100				60		10.4
100				60		13.0
100	168			40		3213.6
100	168			40		3857.1
100		168		40		10.4
100		168		40		14.3
100	168		25	20	90	5255.9
100	168		25		90	4755.4
100		168	25	20	90	6.5
100		168		20	90	1.4

Table2

Blood mocroliter	h 20-44 pep-tide microgram/ml	F19A5B4 microgram/ml	0.1%BSA	PGN microgram/ml	IL-6 pg/ml
100	360		40		510.5
100	360		40		366.6
100	180		40		122.7
100	180		40		142.7
100	90		40		60.7
100	90		40		51.9
100			40	250	15331.1
100			40	250	13856.5
100	360		20	250	27639.4
100	360		20	250	23178.0
100	180		20	250	19289.1
100	180		20	250	22415-3
100	90		20	250	14668.9
100	90		20	250	8109.7
100	45		20	250	10619.0
100	45		20	250	9783.6

100	360	168	20		317.4
100	360	168	20		255.7
100	180	168	20		80.7
100	180	168	20		70.6
100	90	168	20		56.8
100	90	168	20		60.7
100	45	168	20		17.3
100	45	168	20		16.4
100	360	168		250	3638.7
100	360	168		250	2815.7
100	180	168		250	730.1
100	180	168		250	1006.4
100	90	168		250	337.7
100	90	168		250	145.0
100	45	168		250	60.1

From table 1 it appears that the monoclonal antibody produced by clone F19A5B1 (fusion No19 well A5 sub-clonet and well B1 selected) enhance the IL-6 secretion when added alone and potentiate the IL-6 production when added together with PCW and pHBP in the whole blood assay described in example 1. From the same table it also appears that the monoclonal antibody F19A5B4, obtained from well 4 in the same sub-cloning of F19A5, has the opposite effect in a total inhibition of the IL-6 production when added either alone of in the presence of PCW and pHBP.

From table 2 it appears that h 20-44 peptide acetylated in the N terminal and amidated in the C terminal respectively enhance the IL-6 secretion when added alone and enhance the IL-6 secretion when added together with PGN. However, the monoclonal antibody F19A5B4 inhibit these effects of the substituted h 20-44 peptide.

Conclusion

Highly specific monoclonal and also polyclonal antibodies raised against HBP or peptides thereof can be produced and selected to stimulate the immune response (measured as IL-6 secretion but not restricted to this cytokine as a number of other

cytokines such as TNF-alpha, IL-1, IL-8 can be measured). The antibodies potentiate the immune response induces by bacterial products

Highly specific monoclonal and also polyclonal antibodies raised against HBP or peptides thereof can be produced and selected for inhibition of the immune response (measured as IL-6 secretion but not restricted to this cytokine as a number of other cytokines such as TNT-alpha, IL-1, IL-6 can be measured) The antibodies inhibit the immune response induced by bacterial products. The antibodies inhibit the immune response induces by endogenous HBP or HBP and peptides thereof, specifically substituted h 20-44 added exogenously. The antibodies inhibit the amplification of the immune response induces by endogenous HBP or HBP or peptides thereof added exogenously together with bacterial products.

Claims

1. A peptide having a sequence of at most 44 amino acid residues comprising a motif of the formula

X^1-X^2-Cys-X^3-X^4-X^5-X^6-X^7-X^8-X^9-X^{10}-X^{11}-X^{12}-X^{13}-X^{14}-X^{15}-X^{16}-X^{17}-Cys-X^{18}-X^{19},

wherein

X can be an amino acid sequence or a single amino acid residue selected either from Group 1 consisting of Ala, Gly, and Ser,

Group 2 consisting of Arg and Lys,

Group 3 consisting of His, Ile, Leu, Met, Phe, Pro, Thr, Val, Trp, and Tyr,

Group 4 consisting of Asn and Gln , or

Group 5 consisting of Ala, Asn, Arg, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val,

wherein

X^1 can be represented by a sequence consisting of 2-5 amino acid residues or an amino acid residue selected from Group 2;

X^2 is selected from Group 5 or Group 3;

X^3, X^{15} and X^4 are selected from Group 1;

X^5 is Thr or selected from from Group 1;

X^6, X^{11}, X^{12}, X^{13} and X^7 are selected from Group 3;

X^8 and X^{17} are selected from Group 1, 3 or 4;

X^9 is selected from from Group 5, 1 or 3;

X^{10} is selected from from Group 2, 3 or 4;

X^{14} is Ser or selected from from Group 3;

X^{16} and X^{18} is selected from Group 1 or 3

X^{19} can be represented by a sequence consisting of 2-5 amino acid residues or a single amino acid residue selected from Group 5, 2, or 4,

with the proviso, that when X^1 includes Pro, then X^{19} is Gln.

2. The peptide according to claim 1, wherein X^1 is represented by an amino acid sequence selected from SEQ ID NOS: 607-612.

3. The peptide according to claim 1 or 2, wherein X^1 is Arg.

4. The peptide according to any of the claims 1-5, wherein X^2 is Phe.

5. The peptide according to any of the claims 1-6, wherein X^3 is Ala or Gly.

6. The peptide according to any of the claims 1-7, wherein X^4 is Gly.

7. The peptide according to any of the claims 1-8, wherein X^5 is Ala.

8. The peptide according to any of the claims 1-9, wherein X^6 is Leu.

9. The peptide according to any of the claims 1-10, wherein X^7 is Ile, Leu, Met or Val.

10. The peptide according to any of the claims 1-11, wherein X^8 is His or Val.

11. The peptide according to any of the claims 1-12, wherein X^9 is Ala, Phe or Pro.

12. The peptide according to any of the claims 1-13, wherein X^{10} is Arg.

13. The peptide according to any of the claims 1-14, wherein X^{11} is Phe or Pro.

14. The peptide according to any of the claims 1-15, wherein X^{12} is His or Val.

15. The peptide according to any of the claims 1-16, wherein X^{13} is Ile, Leu, Met or Val.

16. The peptide according to any of the claims 1-17, wherein X^{14} is Thr.

17. The peptide according to any of the claims 1-18, wherein X^{15} is Ala.

18. The peptide according to any of the claims 1-19, wherein X^{16} is Ala.

19. The peptide according to any of the claims 1-20, wherein X^{17} is Ser.

20. The peptide according to any of the claims 1-21, wherein X^{18} is Phe.

21. The peptide according to any of the claims 1-22, wherein X^{19} is a sequence identified as SEQ ID NO: 613

22. The peptide according to claims any of the claims 1-23, wherein X^{19} is Arg or Gln.

23. The peptide according to claim 3, wherein the N-terminal of the polypeptide chain is modified.

24. The peptide according to claim 4, wherein the N-terminal amino group is acetylated.

25. The peptide according to claim 24, wherein the C-terminal of the polypeptide chain is modified.

26. The peptide according to claim 25, wherein the C-terminal carboxy group is amidated.

27. The peptide according to any of the claims 1-26, wherein said peptide has the sequence KQGRHFCGGALIHARFVMTAASCFR (SEQ ID NO: 595).

28. The peptide according to any of the claims 1-26, wherein said peptide has the sequence KQGRPFCGGALIHARFVMTAASCFR (SEQ ID NO: 596).

29. The peptide according to any of the claims 1-26, wherein said peptide has the sequence KQGRHFCGGALIHPRFVMTAASCFR (SEQ ID NO: 597).

30. The peptide according to any of the claims 1-26, wherein said peptide has the sequence KQGRPFCGGALIHPRFVMTAASCFR (SEQ ID NO: 598).

31. The peptide according to any of the claims 1-26, wherein said peptide has the sequence RFCSAATLVFRPHVLAGACFPRGQK (SEQ ID NO: 599).

32. The peptide according to any of the claims 1-26, wherein said peptide has the sequence NQGRPFCAGALVHPRFVLTAASCFR (SEQ ID NO: 600).

33. The peptide according to any of the claims 1-26, wherein said peptide has the sequence KQGRPFCAGALVHPRFVLTAASCFQ (SEQ ID NO: 601).

34. The peptide according to any of the claims 1-26, wherein said peptide has the sequence NQGRPFCAGALVHPRFVLTAASCFQ (SEQ ID NO: 602).

35. The peptide according to any of the claims 1-26, wherein said peptide has the sequence KQGRPFCAGALVHPQFVLTAASCFR (SEQ ID NO: 603).

36. The peptide according to any of the claims 1-26, wherein said peptide has the sequence LRGGHFCGATLIAPNFVMSAAHCVA (SEQ ID NO: 604).

37. The peptide according to any of the claims 1-26, wherein said peptide has the sequence RRGGHFCGATLIARNFVMSAVHCVN (SEQ ID NO: 605).

38. The peptide according to any of the claims 1-26, wherein said peptide has the sequence RSREYRCGGTLVSQRYILTAASCAA (SEQ ID NO: 606).

39. The peptide according to any of the claims 1-26, wherein said peptide has the sequence NQGRHFCGGALIHARFVMTAASCFQ (SEQ ID NO: 594).

40. The peptide according to any of the claims 1-26, wherein said peptide has the sequence KQGRPFCAGALVHPRFVLTAASCFR (SEQ ID NO: 593).

41. The peptide according to claim 39 or 40, wherein the C-terminal carboxy group of said peptide is amidated and/or the N-terminal amino group is acetylated.

42. The peptide according to claim 1, wherein the motif comprises a sequence of the formula Cys-$X_{(11)}$-Ala-Ala-Ser-Cys, wherein $X_{(11)}$-represents a contiguous sequence of 11 amino acids.

43. The peptide according to claims 1, 2 or 42, wherein the sequence comprises one or more amino acid sequences are selected from SEQ ID NOS: 12-39.

44. The peptide according to claims 1-26 or 42 wherein the sequence comprises one or more amino acid sequences are selected from SEQ ID NOS: 233-253.

45. The peptide according to claims 1-26 or 42 and 44, wherein the sequence comprises one or more amino acid sequences are selected from SEQ ID NOS: 233-253 and SEQ ID NO: 34.

46. The peptide according to claims 1-26 or 42 and 44-45, wherein the sequence comprises one or more amino acid sequences are selected from SEQ ID NOS: 233-253 and SEQ ID NO: 21.

47. The peptide according to claims 1-26 or 42 and 44-45, wherein the sequence comprises one or more amino acid sequences are selected from SEQ ID NOS: 233-253 and SEQ ID NO: 21 and SEQ ID NO: 34.

48. The peptide according to claims 1-26 and 42, wherein the sequence comprises one or more amino acid sequences are selected from SEQ ID NOS: 395-421.

49. The peptide according to claims 1-26 and 42, wherein the one or more amino acid sequences are derived from the sequence of human heparin-binding protein (hHBP) set forth in SEQ ID NO: 1.

50. The peptide according to claims 1-26 and 42, wherein the one or more amino acid sequences are derived from the sequence of porcine heparin-binding protein (pHBP) set forth in SEQ ID NO: 588.

51. The peptide according to claims 1-26 and 42, wherein the one or more amino acid sequences are derived from the sequence of human neutrophil elastase set forth in SEQ ID NO: 589.

52. The peptide according to claims 1-26 and 42 comprising one or more amino acid sequences set forth in SEQ ID NOS: 2-587.

53. The peptide according to any of the claims 1-52, wherein said peptide is capable of inhibiting the secretion of cytokine IL-6 from monocytes.

54. The peptide according to any of the claims 1-52, wherein said peptide is capable of stimulating the secretion of cytokine IL-6 from monocytes.

55. A process for the production of a peptide as defined in any of the claims 1-54, comprising the steps of

a) providing an expression vector containing a DNA sequence encoding one or more of the amino acid sequences as defined in the claims 1-54,

b) transforming host cells with the vector of step (a);

c) culturing the transformed cells of step (b);

d) purifying the expressed peptide.

56. The process according to claim 55, wherein the host cells are selected from the group comprising recombinant bacterial, yeast, insect or mammalian cells.

57. Use of one or more peptides as defined in any of the claims 1-54 for the manufacture of a medicament for the treatment of Gram negative bacterial infection.

58. Use of one or more peptides as defined in any of the claims 1-54 for the manufacture of a medicament for the treatment of Gram positive bacterial infection.

59. Use of one or more peptides as defined in any of the claims 1-54 for the manufacture of a medicament for the treatment of sepsis, severe sepsis, septic shock and disseminated intravascular coagulation.

60. Use of one or more peptides as defined in any of the claims 1-54 for the manufacture of a medicament for the treatment of meningitis.

61. The use according to claim 58, wherein meningitis is meningococcal meningitis.

62. Use of one or more peptides as defined in any of the claims 1-54 for the manufacture of a medicament for the treatment of pneumonia.

63. The use according to claim 60, wherein pneumonia is pneumococcal pneumonia.

64. Use of one or more peptides as defined in any of the claims 1-52 and 54 for the manufacture of a medicament for the stimulation of inflammatory response.

65. Use of one or more peptides as defined in any of the claims 1-53 for the manufacture of a medicament for the inhibition of inflammatory response.

66. Use of one or more peptides as defined in any of the claims 1-54 for the manufacture of a medicament for the prevention of cell apoptosis.

67. Use of a peptide comprising two or more sequences set forth in SEQ ID NOS: 20-36, wherein said sequences constitute a contiguous sequence derived from the sequence of human HBP set forth in SEQ ID NO:1 for the manufacture of a medicament for the treatment of individuals having suppressed immune system, cancer, auto-immune diseases and/or trauma.

68. The use according to claim 67, wherein the peptide is having the sequence identified in SEQ ID NO: 594, fragments, or variants thereof.

69. The use according to claim 68, wherein the peptide has the N-terminal amino group acetylated and/or C-terminal carboxy group amidated.

70. Use of a peptide comprising the sequence comprises one or more amino acid sequences are selected from SEQ ID NOS: 233-253 and SEQ ID NO: 21 and SEQ ID NO: 34, wherein said sequences constitute a contiguous sequence derived from the sequence of porcine HBP set forth in SEQ ID NO: 588 for the manufacture of a medicament for the treatment of individuals to suppress a sustained inflammatory response.

71. The use according to claim 70, wherein the peptide is having the sequence identified in SEQ ID NO: 593, fragments, or variants thereof.

72. The use according to claim 71, wherein the peptide has the N-terminal amino group acetylated and/or C-terminal carboxy group amidated.

73. Use of an antibody directed against an epitope comprising one or more of the sequences set forth in SEQ ID NO: SEQ ID NOS: 12-39, 233-253, 395-421, said antibody is capable of inhibiting the inflammatory response associated with one or more biological activities of hHBP or a hHBP fragment comprising amino acids 20-44 according to the sequence identified as SEQ ID NO: 1, for the manufacture of a medicament for pathological conditions wherein inhibition of inflammatory response is required.

74. The use according to claim 73, wherein the antibody is polyclonal.

75. The use according to claim 73, wherein the antibody is monoclonal.

76. The use according to claim 75, wherein the antibody is F19A5B4.

77. Use of an antibody directed against an epitope comprising one or more of the sequences set forth in SEQ ID NO: 2-11, 40-232, 254-394, 421-587, said antibody is capable of stimulating the inflammatory response associated with one or more biological activities of hHBP or a hHBP fragment comprising amino acids 20-44 according to the sequence identified as SEQ ID NO: 1, for the

manufacture of a medicament for pathological conditions wherein stimulation of inflammatory response is required.

78. The use according to claim 75, wherein the antibody is polyclonal.

79. The use according to claim 75, wherein the antibody is monoclonal.

80. The use according to claim 77, wherein the antibody is F19A5B1.

81. A medicament comprising a peptide as defined in claims 1-54 and/or an antibody as defined in claims 73-80.

Modtaget
27 JUNI 2003
PVS

Sequence listing

SEQ ID NO:1 mature human HBP: 1-225aa

IVGGRKARPRQFQFLASIQNQGRHFCGGALIHARFVMTAASCFQSQNPGV STVVLGAYDL
RRRERQSRQTFSISSMSENGYDPQQNLNDLMLLQLDREANLTSSVTILPL PLQNATVEAG
TRCQVAGWGSQRSGGRLSRFPRFVNVTVTPEDQCRPNNVCTGVLTRRGGICNGDGGTPLVCEG
LAHGVASFSLGPCGRGPDFFTRVALFRDWIDGVLNNPGPGPA

SEQ ID NO:2 : ARPR (7-10);
SEQ ID NO:3 RPRQ (8-11);
SEQ ID NO:4 PRQF (9-12);
SEQ ID NO:5 RQFQ (10-13);
SEQ ID NO:6 QFQF (11-14);
SEQ ID NO:7 FQFL (12-15);
SEQ ID NO:8 QFLA (13-16);
SEQ ID NO:9 FLAS (14-17);
SEQ ID NO:10 LASI (15-18);
SEQ ID NO:11 ASIQ (16-19);
SEQ ID NO:12 SIQN (17-20);
SEQ ID NO:13 IQNQ (18-21);
SEQ ID NO:14 QNQG (19-22);
SEQ ID NO:15 NQGR (20-23);
SEQ ID NO:16 QGRH (21-24);
SEQ ID NO:17 GRHF (22-25);
SEQ ID NO:18 RHFC (23-26);
SEQ ID NO:19 HFCG (24-27);
SEQ ID NO:20 FCGG (25-28);
SEQ ID NO:21 CGGA (26-29);
SEQ ID NO:22 GGAL (27-30);
SEQ ID NO:23 GALI (28-31);
SEQ ID NO:24 ALIH (29-32);
SEQ ID NO:25 LIHA (30-33);
SEQ ID NO:26 IHAR (31-34);
SEQ ID NO:27 HARF (32-35);
SEQ ID NO:28 ARFV (33-36);
SEQ ID NO:29 RFVM (34-37);
SEQ ID NO:30 FVMT (35-38);

SEQ ID NO:31 VMTA (36-39);
SEQ ID NO:32 MTAA (37-40);
SEQ ID NO:33 TAAS (38-41);
SEQ ID NO:34 AASC (39-42);
SEQ ID NO:35 ASCF (40-43);
SEQ ID NO:36 SCFQ (41-44);
SEQ ID NO:37 CFQS (42-45);
SEQ ID NO:38 FQSQ (43-46);
SEQ ID NO:39 QSQN (44-47);
SEQ ID NO:40 SQNP (45-48);
SEQ ID NO:41 QNPG (46-49);
SEQ ID NO:42 NPGV (47-50);
SEQ ID NO:43 PGVS (48-51);
SEQ ID NO:44 GVST (49-52);
SEQ ID NO:45 VSTV (50-53);
SEQ ID NO:46 STVV (51-54);
SEQ ID NO:47 TVVL (52-55);
SEQ ID NO:48 VVLG (53-56);
SEQ ID NO:49 VLGA (54-57);
SEQ ID NO:50 LGAY (55-58);
SEQ ID NO:51 GAYD (56-59);
SEQ ID NO:52 AYDL (57-60);
SEQ ID NO:53 YDLR (58-61);
SEQ ID NO:54 DLRR (59-62);
SEQ ID NO:55 LRRR (60-63);
SEQ ID NO:56 RRRE (61-64);
SEQ ID NO:57 RRER (62-65);
SEQ ID NO:58 RERQ (63-66);
SEQ ID NO:59 ERQS (64-67);
SEQ ID NO:60 RQSR (65-68);
SEQ ID NO:61 QSRQ (66-69);
SEQ ID NO:62 SRQT (67-70);
SEQ ID NO:63 RQTF (68-71);
SEQ ID NO:64 QTFS (69-72;
SEQ ID NO:65 TFSI (70-73);

SEQ ID NO:66 FSIS (71-74);
SEQ ID NO:67 SISS (72-75)
SEQ ID NO:68 ISSM (73-76);
SEQ ID NO:69 SSMS (74-77);
SEQ ID NO:70 SMSE (75-78);
SEQ ID NO:71 MSEN (76-79);
SEQ ID NO:72 SENG (77-80);
SEQ ID NO:73 ENGY (78-81);
SEQ ID NO:74 NGYD (79-82);
SEQ ID NO:75 GYDP (80-83);
SEQ ID NO:76 YDPQ (81-84);
SEQ ID NO:77 DPQQ (82-85);
SEQ ID NO:78 PQQN (83-86);
SEQ ID NO:79 QQNL (84-87);
SEQ ID NO:80 QNLN (85-88);
SEQ ID NO:81 NLND (86-89);
SEQ ID NO:82 LNDL (87-90);
SEQ ID NO:83 NDLM (88-91);
SEQ ID NO:84 DLML (89-92);
SEQ ID NO:85 LMLL (90-93);
SEQ ID NO:86 MLLQ (91-94);
SEQ ID NO:87 LLQL (92-95);
SEQ ID NO:88 LQLD (93-96);
SEQ ID NO:89 QLDR (94-97);
SEQ ID NO:90 LDRE (95-98);
SEQ ID NO:91 DREA (96-99);
SEQ ID NO:92 REAN (97-100);
SEQ ID NO:93 EANL (98-101);
SEQ ID NO:94 ANLT (99-102);
SEQ ID NO:95 NLTS (100-103);
SEQ ID NO:96 LTSS (101-104);
SEQ ID NO:97 TSSV (102-105);
SEQ ID NO:98 SSVT (103-106);
SEQ ID NO:99 SVTI (104-107);
SEQ ID NO:100 VTIL (105-108);

SEQ ID NO:101 TILP (106-109);
SEQ ID NO:102 ILPL (107-110);
SEQ ID NO:103 LPLP (108-111);
SEQ ID NO:104 PLPL (109-112);
SEQ ID NO:105 LPLQ (110-113);
SEQ ID NO:106 PLQN (111-114);
SEQ ID NO:107 LQNA (112-115);
SEQ ID NO:108 QNAT (113-116);
SEQ ID NO:109 NATV (114-117);
SEQ ID NO:110 ATVE (115-118);
SEQ ID NO:111 TVEA (116-119);
SEQ ID NO:112 VEAG (117-120);
SEQ ID NO:113 EAGT (118-121);
SEQ ID NO:114 AGTR (119-122);
SEQ ID NO:115 GTRC (120-123);
SEQ ID NO:116 TRCQ (121-124);
SEQ ID NO:117 RCQV (122-125);
SEQ ID NO:118 CQVA (123-126);
SEQ ID NO:119 QVAG (124-127);
SEQ ID NO:120 VAGW (125-128);
SEQ ID NO:121 AGWG (126-129);
SEQ ID NO:122 GWGS (127-130);
SEQ ID NO:123 WGSQ (128-131);
SEQ ID NO:124 GSQR (129-132);
SEQ ID NO:125 SQRS (130-133);
SEQ ID NO:126 QRSG (131-134);
SEQ ID NO:127 RSGG (132-135);
SEQ ID NO:128 SGGR (133-136);
SEQ ID NO:129 GGRL (134-137);
SEQ ID NO:130 GRLS (135-138);
SEQ ID NO:131 RLSR (136-139);
SEQ ID NO:132 LSRF (137-140);
SEQ ID NO:133 SRFP (138-141);
SEQ ID NO:134 RFPR (139-142);
SEQ ID NO:135 FPRF (140-143);

SEQ ID NO:136 PRFV (141-144);
SEQ ID NO:137 RFVN (142-145);
SEQ ID NO:138 FVNV (143-146);
SEQ ID NO:139 VNVT (144-147);
SEQ ID NO:140 NVTV (145-148);
SEQ ID NO:141 VTVT (146-149);
SEQ ID NO:142 TVTP (147-150);
SEQ ID NO:143 VTPE (148-151);
SEQ ID NO:144 TPED (149-152);
SEQ ID NO:145 PEDQ (150-153);
SEQ ID NO:146 EDQC (151-154);
SEQ ID NO:147 DQCR (152-155);
SEQ ID NO:148 QCRP (153-156);
SEQ ID NO:149 CRPN (154-157);
SEQ ID NO:150 RPNN (155-158);
SEQ ID NO:151 PNNV (156-159);
SEQ ID NO:152 NNVC (157-160);
SEQ ID NO:153 NVCT (158-161);
SEQ ID NO:154 VCTG (159-162);
SEQ ID NO:155 CTGV (160-163);
SEQ ID NO:156 TGVL (161-164);
SEQ ID NO:157 GVLT (162-165);
SEQ ID NO:158 VLTR (163-166);
SEQ ID NO:159 LTRR (164-167);
SEQ ID NO:160 TRRG (165-168);
SEQ ID NO:161 RRGG (166-169);
SEQ ID NO:162 RGGI (167-170);
SEQ ID NO:163 GGIC (168-171);
SEQ ID NO:164 GICN (169-172);
SEQ ID NO:165 ICNG (170-173);
SEQ ID NO:166 CNGD (171-174);
SEQ ID NO:167 NGDG (172-175);
SEQ ID NO:168 GDGG (173-176);
SEQ ID NO:169 DGGT (174-177);
SEQ ID NO:170 GGTP (175-178);

SEQ ID NO:171 GTPL (176-179),
SEQ ID NO:172 TPLV (177-180);
SEQ ID NO:173 PLVC (178-181);
SEQ ID NO: 174 LVCE (179-181)
SEQ ID NO:175 VCEG (180-183);
SEQ ID NO:176 CEGL (181-184);
SEQ ID NO:177 EGLA (182-185);
SEQ ID NO:178 GLAH (183-186);
SEQ ID NO:179 LAHG (184-187);
SEQ ID NO:180 AHGV (185-188);
SEQ ID NO:181 HGVA (186-189);
SEQ ID NO:182 GVAS (187-190);
SEQ ID NO:183 VASF (188-191);
SEQ ID NO:184 ASFS (189-192);
SEQ ID NO:185 SFSL (190-193);
SEQ ID NO:186 FSLG (191-194);
SEQ ID NO:187 SLGP (192-195);
SEQ ID NO:188 LGPC (193-196);
SEQ ID NO:189 GPCG (194-197);
SEQ ID NO:190 PCGR (195-198);
SEQ ID NO:191 CGRG (196-199);
SEQ ID NO:192 GRGP (197-200);
SEQ ID NO:193 RGPD (198-201);
SEQ ID NO:194 GPDF (199-202);
SEQ ID NO:195 PDFF (200-203);
SEQ ID NO:196 DFFT (201-204);
SEQ ID NO:197 FFTR (202-205);
SEQ ID NO:198 FTRV (203-206);
SEQ ID NO:199 TRVA (204-207);
SEQ ID NO:200 RVAL (205-208);
SEQ ID NO:201 VALF (206-209);
SEQ ID NO:202 ALFR (207-210);
SEQ ID NO:203 LFRD (208-211);
SEQ ID NO:204 FRDW (209-212);
SEQ ID NO:205 RDWI (210-213);

SEQ ID NO:206 DWID (211-214);
SEQ ID NO:207 WIDG (212-215);
SEQ ID NO:208 IDGV (213-216);
SEQ ID NO:209 DGVL (214-217);
SEQ ID NO:210 GVLN (215-218);
SEQ ID NO:211 VLNN (216-219);
SEQ ID NO:212 LNNP (217-220);
SEQ ID NO:213 NNPG (218-221);
SEQ ID NO:214 NPGP (219-222);
SEQ ID NO:215 PGPG (220-223);
SEQ ID NO:216 GPGP (221-224);
SEQ ID NO:217 PGPA (222-225);
SEQ ID NO:218 GGRK (3-6);
SEQ ID NO:219 GRKA (4-7);
SEQ ID NO:220 RKAR (5-8);
SEQ ID NO:221 KARP (6-9);

pHBP peptide sequences differing from hHBP

SEQ ID NO:222 GGRR (3-6)
SEQ ID NO:223 GRRA (4-7)
SEQ ID NO:224 RRAQ (5-8)
SEQ ID NO:225 RAQP (6-9)
SEQ ID NO:226 AQPQ (7-10)
SEQ ID NO:227 QPQE (8-11)
SEQ ID NO:228 PQEF (9-12)
SEQ ID NO:229 QEFP (10-13)
SEQ ID NO:230 EFPF (11-14)
SEQ ID NO:231 FPFL (12-15)
SEQ ID NO:232 PFLA (13-16)
SEQ ID NO:233 QGRP (21-24)
SEQ ID NO:234 GRPF (22-25)
SEQ ID NO:235 RPFC (23-26)
SEQ ID NO:236 PFCA (24-27)
SEQ ID NO:237 FCAG (25-28)

SEQ ID NO:238 CAGA (26-29)
SEQ ID NO:239 AGAL (27-30)
SEQ ID NO:240 GALV (28-31)
SEQ ID NO:241 ALVH (29-32)
SEQ ID NO:242 LVHP (30-33)
SEQ ID NO:243 VHPR (31-34)
SEQ ID NO:244 HPRF (32-35)
SEQ ID NO:245 SIQK (17-20)
SEQ ID NO:246 RFVL (34-37)
SEQ ID NO:247 FVLT (35-38)
SEQ ID NO:248 VLTA (36-39)
SEQ ID NO:249 LTAA (37-40)
SEQ ID NO:250 SCFR (41-44)
SEQ ID NO:251 CFRG (42-45)
SEQ ID NO:252 FRGK (43-46)
SEQ ID NO:253 RGKN (44-47)
SEQ ID NO:254 GKNS (45-48)
SEQ ID NO:255 KNSG (46-49)
SEQ ID NO:256 NSGS (47-50)
SEQ ID NO:257 SGSA (48-51)
SEQ ID NO:258 GSAS (49-52)
SEQ ID NO:259 SASV (50-53)
SEQ ID NO:260 ASVV (51-54)
SEQ ID NO:261 SVVL (52-55)
SEQ ID NO:262 DLRQ (59-62)
SEQ ID NO:263 LRQQ (60-63)
SEQ ID NO:264 RQQE (61-64)
SEQ ID NO:265 QQEQ (62-65)
SEQ ID NO:266 QEQS (63-66)
SEQ ID NO:267 EQSR (64-67)
SEQ ID NO:268 IQKQ (18-21)
SEQ ID NO:269 FSIR (70-73)
SEQ ID NO:270 SIRS (71-74)
SEQ ID NO:271 IRSI (72-75)
SEQ ID NO:272 RSIS (73-76)

SEQ ID NO:273 SISQ (74-77)
SEQ ID NO:274 ISQN (75-78)
SEQ ID NO:275 SQNG (76-79)
SEQ ID NO:276 QNGY (77-80)
SEQ ID NO:277 YDPR (80-83)
SEQ ID NO:278 DPRQ (81-84)
SEQ ID NO:279 PRQN (82-85)
SEQ ID NO:280 RQNL (83-86)
SEQ ID NO:281 LNDV (86-89)
SEQ ID NO:282 NDVL (87-90)
SEQ ID NO:283 DVLL (88-91)
SEQ ID NO:284 VLLL (89-92)
SEQ ID NO:285 LLLQ (90-93)
SEQ ID NO:286 REAR (96-99)
SEQ ID NO:287 EARL (97-100)
SEQ ID NO:288 ARLT (98-101)
SEQ ID NO:289 RLTP (99-102)
SEQ ID NO:290 LTPS (100-103)
SEQ ID NO:291 TPSV (101-104)
SEQ ID NO:292 PSVA (102-105)
SEQ ID NO:293 SVAL (103-106)
SEQ ID NO:294 VALV (104-107)
SEQ ID NO:295 ALVP (105-108)
SEQ ID NO:296 LVPL (106-109)
SEQ ID NO:297 VPLP (107-110)
SEQ ID NO:298 PLPP (108-111)
SEQ ID NO:299 LPPQ (109-112)
SEQ ID NO:300 PPQN (110-113)
SEQ ID NO:301 PQNA (111-114)
SEQ ID NO:302 AGTN (112-115)
SEQ ID NO:303 GTNC (113-116)
SEQ ID NO:304 TNCQ (114-117)
SEQ ID NO:305 NCQV (121-124)
SEQ ID NO:306 GWGT (126-129)
SEQ ID NO:307 WGTQ (127-130)

SEQ ID NO:308 GTQR (128-131)
SEQ ID NO:309 TQRL (129-132)
SEQ ID NO:310 QRLR (130-133)
SEQ ID NO:311 RLRR (131-134)
SEQ ID NO:312 LRRL (132-135)
SEQ ID NO:313 RRLF (133-136)
SEQ ID NO:314 RLFS (134-137)
SEQ ID NO:315 LFSR (135-138)
SEQ ID NO:316 FSRF (136-139)
SEQ ID NO:317 FPRV (139-142)
SEQ ID NO:318 PRVL (140-143)
SEQ ID NO:319 RVLN (141-144)
SEQ ID NO:320 VLNV (142-145)
SEQ ID NO:321 LNVT (143-146)
SEQ ID NO:322 TVTS (146-149)
SEQ ID NO:323 VTSN (147-150)
SEQ ID NO:324 TSNP (148-151)
SEQ ID NO:325 SNPC (149-152)
SEQ ID NO:326 NPCL (150-153)
SEQ ID NO:327 PCLP (151-154)
SEQ ID NO:328 CLPR (152-155)
SEQ ID NO:329 LPRD (153-156)
SEQ ID NO:330 PRDM (154-157)
SEQ ID NO:331 RDMC (155-158)
SEQ ID NO:332 DMCI (156-159)
SEQ ID NO:333 MCIG (157-160)
SEQ ID NO:334 CIGV (158-161)
SEQ ID NO:335 IGVF (159-162)
SEQ ID NO:336 GVFS (160-163)
SEQ ID NO:337 VFSR (161-164)
SEQ ID NO:338 FSRR (162-165)
SEQ ID NO:339 SRRG (163-166)
SEQ ID NO:340 RRGR (164-167)
SEQ ID NO:341 RGRI (165-168)
SEQ ID NO:342 GRIS (166-169)

SEQ ID NO:343 RISQ (167-170)
SEQ ID NO:344 ISQG (168-171)
SEQ ID NO:345 SQGD (169-172)
SEQ ID NO:346 QGDR (170-173)
SEQ ID NO:347 GDRG (171-174)
SEQ ID NO:348 DRGT (172-175)
SEQ ID NO:349 RGTP (173-176)
SEQ ID NO:350 LVCN (177-180)
SEQ ID NO:351 VCNG (178-181)
SEQ ID NO:352 CNGL (179-182)
SEQ ID NO:353 NGLA (180-183)
SEQ ID NO:354 GLAQ (181-184)
SEQ ID NO:355 LAQG (182-185)
SEQ ID NO:356 AQGV (183-186)
SEQ ID NO:357 QGVA (184-187)
SEQ ID NO:358 ASFL (187-190)
SEQ ID NO:359 SFLR (188-191)
SEQ ID NO:360 FLRR (189-192)
SEQ ID NO:361 QKQG (19-22)
SEQ ID NO:362 RRRF (191-194)
SEQ ID NO:363 RRFR (192-195)
SEQ ID NO:364 RFRR (193-196)
SEQ ID NO:365 FRRS (194-197)
SEQ ID NO:366 RRSS (195-198)
SEQ ID NO:367 RSSG (196-199)
SEQ ID NO:368 SSGF (197-200)
SEQ ID NO:369 SGFF (198-201)
SEQ ID NO:370 GFFT (199-202)
SEQ ID NO:371 KQGR (20-23)
SEQ ID NO:372 LFRN (206-209)
SEQ ID NO:373 FRNW (207-210)
SEQ ID NO:374 RNWI (208-211)
SEQ ID NO:375 NWID (209-212)
SEQ ID NO:376 WIDS (210-213)
SEQ ID NO:377 IDSV (211-214)

SEQ ID NO:378 DSVL (212-215)
SEQ ID NO:379 SVLN (213-216)
SEQ ID NO:380 NNPP (216-219)

hNLE peptide sequences differing from hHBP

SEQ ID NO:381 GGRR (3-6)
SEQ ID NO:382 GRRA (4-7)
SEQ ID NO:383 RRAR (5-8)
SEQ ID NO:384 RARP (6-9)
SEQ ID NO:385 ARPH (7-10)
SEQ ID NO:386 RPHA (8-11)
SEQ ID NO:387 PHAW (9-12)
SEQ ID NO:388 HAWP (10-13)
SEQ ID NO:389 AWPF (11-14)
SEQ ID NO:390 WPFM (12-15)
SEQ ID NO:391 PFMV (13-16)
SEQ ID NO:392 FMVS (14-17)
SEQ ID NO:393 MVSL (15-18)
SEQ ID NO:394 VSLQ (16-19)
SEQ ID NO:395 SLQL (17-20)
SEQ ID NO:396 LQLR (18-21)
SEQ ID NO:397 QLRG (19-22)
SEQ ID NO:398 LRGG (20-23)
SEQ ID NO:399 RGGH (21-24)
SEQ ID NO:400 GGHF (22-25)
SEQ ID NO: 401 GHFC (23-26)
SEQ ID NO:402 FCGA (25-28)
SEQ ID NO:403 CGAT (26-29)
SEQ ID NO:404 GATL (27-30)
SEQ ID NO:405 ATLI (28-31)
SEQ ID NO:406 TLIA (29-32)
SEQ ID NO:407 LIAP (30-33)
SEQ ID NO:408 IAPN (31-34)
SEQ ID NO:409 APNF (32-35)

SEQ ID NO:410 PNFV (33-36)
SEQ ID NO:411 NFVM (34-37)
SEQ ID NO:412 FVMS (35-38)
SEQ ID NO:413 VMSA (36-39)
SEQ ID NO:414 MSAA (37-40)
SEQ ID NO:415 SAAH (38-41)
SEQ ID NO:416 AAHC (39-42)
SEQ ID NO:417 AHCV (40-43)
SEQ ID NO:418 HCVA (41-44)
SEQ ID NO:419 CVAN (42-45)
SEQ ID NO:420 VANV (43-46)
SEQ ID NO:421 ANVN (44-47)
SEQ ID NO:422 NVNV (45-48)
SEQ ID NO:423 VNVR (46-49)
SEQ ID NO:424 VRAV (48-51)
SEQ ID NO:425 RAVR (49-52)
SEQ ID NO:426 AVRV (50-53)
SEQ ID NO:427 VRVV (51-54)
SEQ ID NO:428 RVVL (52-55)
SEQ ID NO:429 LGAH (55-58)
SEQ ID NO:430 GAHN (56-59)
SEQ ID NO:431 AHNL (57-60)
SEQ ID NO: 432 HNLS (58-61)
SEQ ID NO:433 NLSR (59-62)
SEQ ID NO:434 LSRR (60-63)
SEQ ID NO:435 SRRE (61-64)
SEQ ID NO:436 RREP (62-65)
SEQ ID NO:437 REPT (63-66)
SEQ ID NO:438 EPTR (64-67)
SEQ ID NO:439 PTRQ (65-68)
SEQ ID NO:440 TRQV (66-69)
SEQ ID NO:441 RQVF (67-70)
SEQ ID NO:442 QVFA (68-71)
SEQ ID NO:443 VFAV (69-72)
SEQ ID NO:444 FAVQ (70-73)

SEQ ID NO:445 AVQR (71-74)
SEQ ID NO:446 VQRI (72-75)
SEQ ID NO:447 QRIF (73-76)
SEQ ID NO:448 RIFE (74-77)
SEQ ID NO:449 IFED (75-78)
SEQ ID NO:450 FEDG (76-79)
SEQ ID NO:451 EDGY (77-80)
SEQ ID NO:452 DGYD (78-81)
SEQ ID NO:453 YDPV (80-83)
SEQ ID NO:454 DPVN (81-84)
SEQ ID NO:455 PVNL (82-85)
SEQ ID NO:456 VNLL (83-86)
SEQ ID NO:457 NLLN (84-87)
SEQ ID NO:458 LLND (85-88)
SEQ ID NO:459 LNDI (86-89)
SEQ ID NO:460 NDIV (87-90)
SEQ ID NO:461 DIVI (88-91)
SEQ ID NO:462 IVIL (89-92)
SEQ ID NO:463 VILQ (90-93)
SEQ ID NO:464 ILQL (91-94)
SEQ ID NO:465 LQLN (92-95)
SEQ ID NO:466 QLNG (93-96)
SEQ ID NO:467 LNGS (94-97)
SEQ ID NO:468 NGSA (95-98)
SEQ ID NO:469 GSAT (96-99)
SEQ ID NO:470 SATI (97-100)
SEQ ID NO:471 ATIN (98-101)
SEQ ID NO:472 TINP (99-102)
SEQ ID NO:473 INPS (100-103)
SEQ ID NO:474 NPSV (101-104)
SEQ ID NO:475 PSVA (102-105)
SEQ ID NO:476 SVAL (103-106)
SEQ ID NO:477 VALV (104-107)
SEQ ID NO:478 ALVP (105-108)
SEQ ID NO:479 LVPL (106-109)

SEQ ID NO:480 VPLP (107-110)
SEQ ID NO:481 PLPA (108-111)
SEQ ID NO:482 LPAQ (109-112)
SEQ ID NO:483 PAQG (110-113)
SEQ ID NO:484 AQGR (111-114)
SEQ ID NO:485 QGRR (112-115)
SEQ ID NO:486 GRRL (113-116)
SEQ ID NO:487 RRLG (114-117)
SEQ ID NO:488 RLGN (115-118)
SEQ ID NO:489 LGNG (116-119)
SEQ ID NO:490 GNGV (117-120)
SEQ ID NO:491 NGVQ (118-121)
SEQ ID NO:492 GVQC (119-122)
SEQ ID NO:493 VQCL (120-123)
SEQ ID NO:494 QCLA (121-124)
SEQ ID NO:495 CLAM (122-125)
SEQ ID NO:496 LAMG (123-126)
SEQ ID NO:497 AMGW (124-127)
SEQ ID NO:498 MGWG (125-128)
SEQ ID NO:499 GWGL (126-129)
SEQ ID NO:500 WGLL (127-130)
SEQ ID NO:501 NVRA (47-50)
SEQ ID NO:502 GLLG (128-131)
SEQ ID NO:503 LLGR (129-132)
SEQ ID NO:504 LGRN (130-133)
SEQ ID NO:505 GRNR (131-134)
SEQ ID NO:506 RNRG (132-135)
SEQ ID NO:507 NRGI (133-136)
SEQ ID NO:508 RGIA (134-137)
SEQ ID NO:509 GIAS (135-138)
SEQ ID NO:510 IASV (136-139)
SEQ ID NO:511 ASVL (137-140)
SEQ ID NO:512 SVLQ (138-141)
SEQ ID NO:513 VLQE (139-142)
SEQ ID NO:514 LQEL (140-143)

SEQ ID NO:515 QELN (141-144)
SEQ ID NO:516 ELNV (142-145)
SEQ ID NO:517 LNVT (143-146)
SEQ ID NO:518 VTVV (145-148)
SEQ ID NO:519 TVVT (146-149)
SEQ ID NO:520 VVTS (147-150)
SEQ ID NO:521 VTSL (148-151)
SEQ ID NO:522 TSLC (149-152)
SEQ ID NO:523 SLCR (150-153)
SEQ ID NO:524 LCRR (151-154)
SEQ ID NO:525 CRRS (152-155)
SEQ ID NO:526 RRSN (153-156)
SEQ ID NO:527 RSNV (154-157)
SEQ ID NO:528 SNVC (155-158)
SEQ ID NO:529 VCTL (157-160)
SEQ ID NO:530 CTLV (158-161)
SEQ ID NO:531 TLVR (159-162)
SEQ ID NO:532 LVRG (160-163)
SEQ ID NO:533 VRGR (161-164)
SEQ ID NO:534 RGRR (162-165)
SEQ ID NO:535 GRRG (163-166)
SEQ ID NO:536 RRGR (164-167)
SEQ ID NO:537 RGRI (165-168)
SEQ ID NO:538 GRIS (166-169)
SEQ ID NO:539 RISQ (167-170)
SEQ ID NO:540 ISQG (168-171)
SEQ ID NO:541 SQGD (169-172)
SEQ ID NO:542 QGDS (170-173)
SEQ ID NO:543 GDSG (171-174)
SEQ ID NO:544 DSGT (172-175)
SEQ ID NO:545 SGTP (173-176)
SEQ ID NO:546 LVCN (177-180)
SEQ ID NO:547 VCNG (178-181)
SEQ ID NO:548 CNGL (179-182)
SEQ ID NO:549 NGLI (180-183)

SEQ ID NO:550 GLIH (181-184)
SEQ ID NO:551 LIHG (182-185)
SEQ ID NO:552 IHGI (183-186)
SEQ ID NO:553 HGIA (184-187)
SEQ ID NO:554 GIAS (185-188)
SEQ ID NO:555 IASF (186-189)
SEQ ID NO:556 ASFV (187-190)
SEQ ID NO:557 SFVR (188-191)
SEQ ID NO:558 FVRG (189-192)
SEQ ID NO:559 VRGG (190-193)
SEQ ID NO:560 RGGC (191-194)
SEQ ID NO:561 GGCA (192-195)
SEQ ID NO:562 GCAS (193-196)
SEQ ID NO:563 CASG (194-197)
SEQ ID NO:564 ASGL (195-198)
SEQ ID NO:565 SGLY (196-199)
SEQ ID NO:566 GLYP (197-200)
SEQ ID NO:567 LYPD (198-201)
SEQ ID NO:568 YPDA (199-202)
SEQ ID NO:569 PDAF (200-203)
SEQ ID NO:570 DAFA (201-204)
SEQ ID NO:571 AFAP (202-205)
SEQ ID NO:572 FAPV (203-206)
SEQ ID NO:573 APVA (204-207)
SEQ ID NO:574 PVAQ (205-208)
SEQ ID NO:575 VAQF (206-209)
SEQ ID NO:576 AQFV (207-210)
SEQ ID NO:577 QFVN (208-211)
SEQ ID NO:578 FVNW (209-212)
SEQ ID NO:579 VNWI (210-213)
SEQ ID NO:580 NWID (211-214)
SEQ ID NO:581 WIDS (212-215)
SEQ ID NO:582 IDSI (213-216)
SEQ ID NO:583 DSII (214-217)
SEQ ID NO:584 SIIQ (214-218)

SEQ ID NO:585 PRFV (33-36)

SEQ ID NO:586 LRRR (190-194)

SEQ ID NO:587 NPPA (217-220)

SEQ ID NO:588 **mature porcine HBP**

~~IVGG RRAQPQEFPF LASIQKQGRP FCAGALVHPR FVLTAASCFR GKNSGSASVV~~
LGAYDLRQQE QSRQTFSIRS ISQNGYDPRQ NLNDVLLLQL DREARLTPSV ALVPLPPQNA
TVEAGTNCQV EAGWGTQRLR R LFSRFPRVLN VTVTSNPCLP RDMCIGVFSR RGRISQGDRG
TPLVCNGLAQ GVASFLRRRF RRSSGFFTRV ALFRNWIDSV LNNPPA

SEQ ID NO:589 **human neutrophil elastase**

MTLGRRLACL FLACVLPALL LGGTALASEI VGGRRARPHA WPFMVSLQLR GGHFCGATLI
APNFVMSAAH CVANVNVRAV RVVLGAHNLS RREPTRQVFA VQRIFENGYD PVNLLNDIVI
LQLNGSATIN ANVQVAQLPA QGRRLGNGVQ CLAMGWGLLG RNRGIASVLQ ELNVTVVTSL
CRRSNVCTLV RGRQAGVCFG DSGSPLVCNG LIHGIASFVR GGCASGLYPD AFAPVAQFVN
WIDSIIQRSE DNPCPHPRDP DPASRTH

SEQ ID NO:590 **cDNA hHBP**

SEQ ID NO: 591 **cDNA pHBP**

AT TGTGGGCGGC AGGAGGGCCC AGCCGCAGGA GTTCCCGTTT CTGGCCTCCA
TTCAGAAACA AGGGAGGCCC TTTTGCGCCG GAGCCCTGGT CCATCCCCGC TTCGTCCTGA
CAGCGGCCAG CTGCTTCCGT GGCAAGAACA GCGGAAGTGC CTCTGTGGTG
CTGGGGGCCT ATGACCTGAG GCAGCAGGAG CAGTCCCGGC AGCATTCTC CATCAGGAGC
ATCAGCCAGA ACGGCTATGA YCCCCGGCAG AATCTGAACG ATGTGCTGCT GCTGCAGCTG
GACCGTGAGG CCGACTCAC CCCCAGTGTG GCCCTGGTAC CGCTGCCCCC GCAGAATGCC
ACAGTGGAAG CTGGCACCAA CTGCCAAGTTGCGGGCTGGG GGACCCAGCG
GCTTAGGAGG CTTTTCTCCC GCTTCCCAAG GGTGCTCAAT GTCACCGTGA CCTCAAACCC
GTGTCTCCCC AGAGCATGT GCATTGGTGT CTTCAGCCGC CGGGGCCGCA TCAGCCAGGG
AGACAGAGGC ACCCCCCTCG TCTGCAACGG CCTGGCGCAG GGCGTGGCCT
CCTTCCTCCG GAGGCGTTTC CGCAGGAGCT CCGGCTTCTT CACCCGCGTG GCGCTCTTCA
GAAATTGGAT TGATTCAGTT CTCAACAACC CGCCGGCCTGA

SEQ ID NO: 592 **cDNA human neutrophil elastase**

CTCGAGAAAA GAATTGTGGG TGGCCGTCGT GCCCGTCCTC ACGCTTGGCC GTTTAT
GGTG TCTCTGCAGC TGCGTGGTGG CCACTTCTGC GGTGCAACCC TGATTGCACC
AAACTTCGTC ATGTCCGCGG CACACTGCGT AGCAAACGTT AACGTTCGTG CGGTGCGTGT
GGTTCTGGGT GCTCATAACC TGTCTCGTCG AGAACCGACC CGTCAAGTGT TCGCCGTGCA
GCGCATCTTC GAAAACGGCT ACGACCCGGT TAACCTGCTG AACGACATCG TGATTCTGCA
ACTGAACGGA TCCGCCACCA TCAACGCCAA CGTGCAAGTG GCACAACTGC
CAGCCCAAGG TCGCCGCCTG GGAAACGGAG TACAATGCCT GGCTATGGGT
TGGGGCCTGC TCGGCCGTAA CCGTGGTATC GCTAGCGTTC TGCAAGAACT GAACGTGACC
GTGGTTACCT CCCTGTGTCG ACGCTCTAAC GTATGCACTC TGGTGCGCGG CCGCCAGGCT
GGCGTTTGTT TCGGTGACTC CGGTAGCCCG CTGGTTTGCA ACGGTCTGAT CCATGGTATT

GCCTCCTTCG TTCGTGGTGG TTGCGCCTCT GGCCTGTACC CGGATGCATT TGCCCCGGTG
GCACAGTTTG TTAACTGGAT CGACTCTATC ATTCAGAGAT CCGAAGACAA CCCGTGTCCG
CACCCACGTG ATCCAGATCC GGCCTCCAGA ACACACCATC ACCATCACCA TTAGGAATTC

SEQ ID NO: 593 **peptide 20-44 of pHBP**

KQGRPFCAGALVHPRFVLTAASCFR (SEQ ID NO: 593)

SEQ ID NO: 594 **peptide 20-44 of hHBP**

NQGRHFCGGALIHARFVMTAASCFQ (SEQ ID NO: 594)

claims 27-39

KQGRHFCGGALIHARFVMTAASCFR (SEQ ID NO: 595) hHBP (20-44) N20K,Q44R
KQGRPFCGGALIHARFVMTAASCFR (SEQ ID NO: 596) hHBP(20-44) N20K,H24P,Q44R
KQGRHFCGGALIHPRFVMTAASCFR (SEQ ID NO: 597) hHBP(20-44) N20K,A33P,Q44R
KQGRPFCGGALIHPRFVMTAASCFR (SEQ ID NO: 598) hHBP(20-44) N20K,H24P,A33P,Q44R
RFCSAATLVFRPHVLAGACFPRGQK (SEQ ID NO: 599) pHBP(44-20), reversed
NQGRPFCAGALVHPRFVLTAASCFR (SEQ ID NO: 600) pHBP(20-44) K20N
KQGRPFCAGALVHPRFVLTAASCFQ (SEQ ID NO: 601) pHBP(20-44) R44Q
NQGRPFCAGALVHPRFVLTAASCFQ (SEQ ID NO: 602) pHBP(20-44) K20N,R44Q
KQGRPFCAGALVHPQFVLTAASCFR (SEQ ID NO: 603) pHBP(20-44) R34Q
LRGGHFCGATLIAPNFVMSAAHCVA (SEQ ID NO: 604) hElastase (20-44)
RRGGHFCGATLIARNFVMSAVHCVN (SEQ ID NO: 605) mElastase (20-44)
RSREYRCGGTLVSQRYILTAASCAA (SEQ ID NO: 606) A. gambiae EAA01962 (20-44)

R^1 amino acid sequences (claim 2)

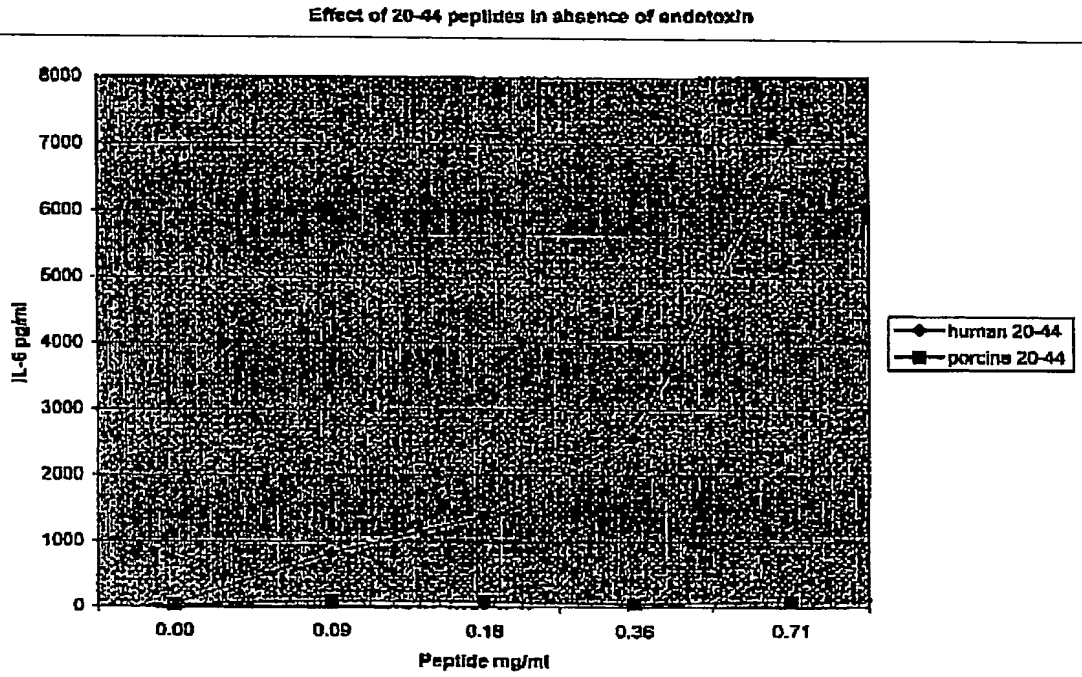
KQGRP (SEQ ID NO: 607) Lys-Gln-Gly-Arg-Pro,
KQGKP (SEQ ID NO: 608) Lys-Gln-Gly-Lys-Pro,
RQGRP (SEQ ID NO: 609) Arg-Gln-Gly-Arg-Pro
RQGKP (SEQ ID NO: 610) Arg-Gln-Gly-Lys-Pro,
NQGRH (SEQ ID NO: 611) Asn-Gln-Gly-Arg-His,
NQGKH (SEQ ID NO: 612) Asn-Gln-Gly-Lys-His

R^{19} amino acid sequences (claim 21)

PRGQK (SEQ ID NO: 613) Pro-Arg-Gly-Gln-Lys

Modtaget
27 JUNI 2003
PVS

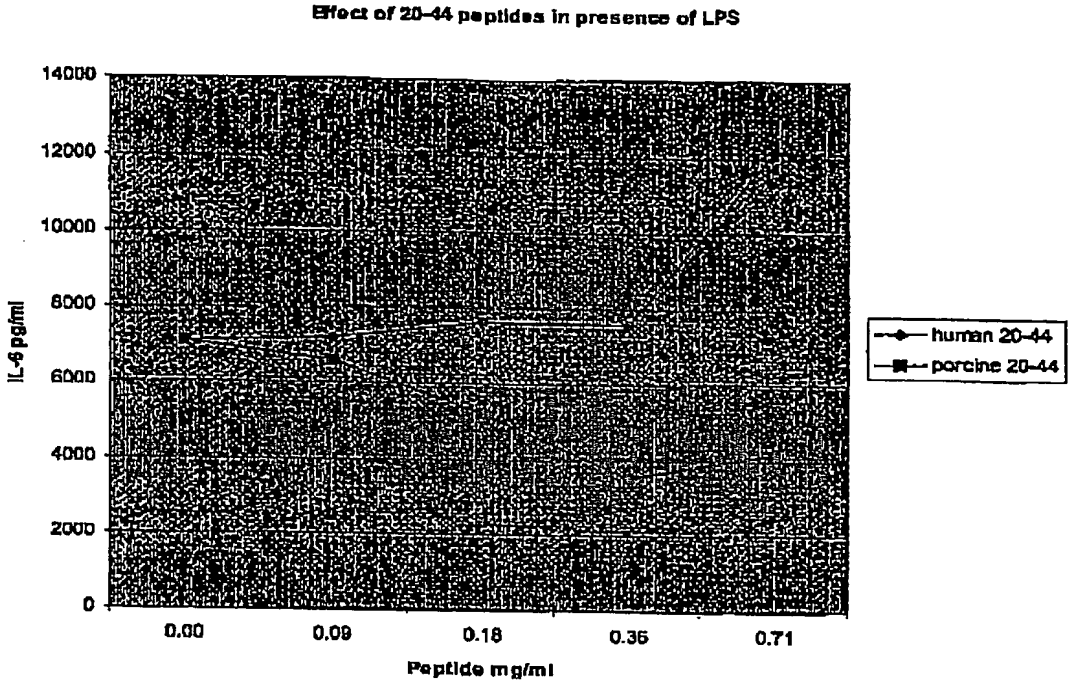
Figure 1.



IL-6 secretion induced by HBP 20-44 peptides in absence of bacterial components

Modtaget
27 JUNI 2003
PVS

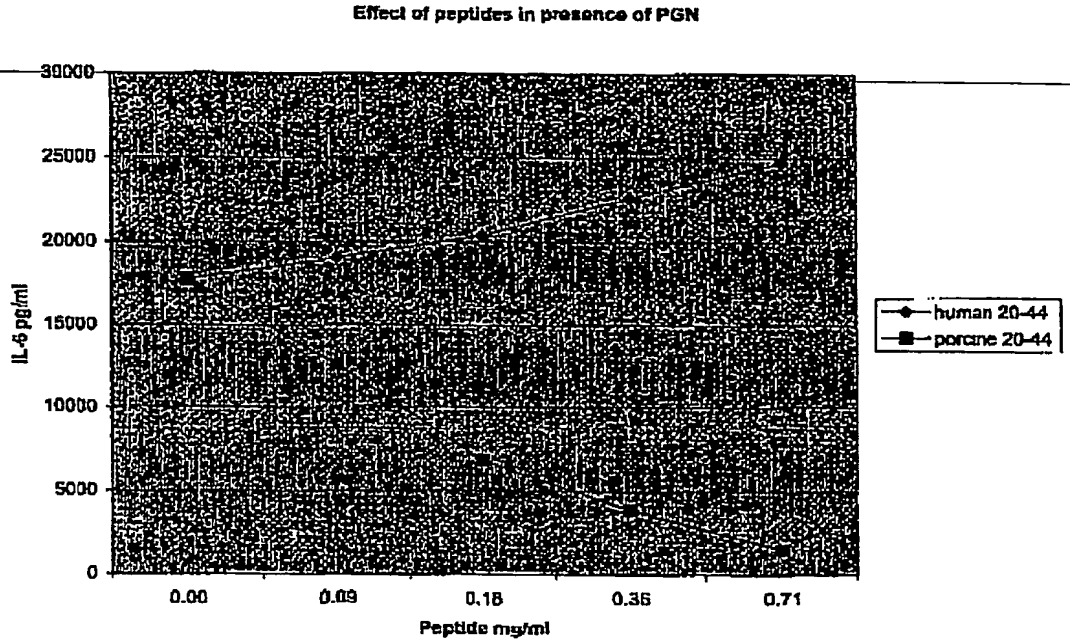
Figure 2



Effect of HBP 20-44 peptides in LPS induced IL-6 secretion

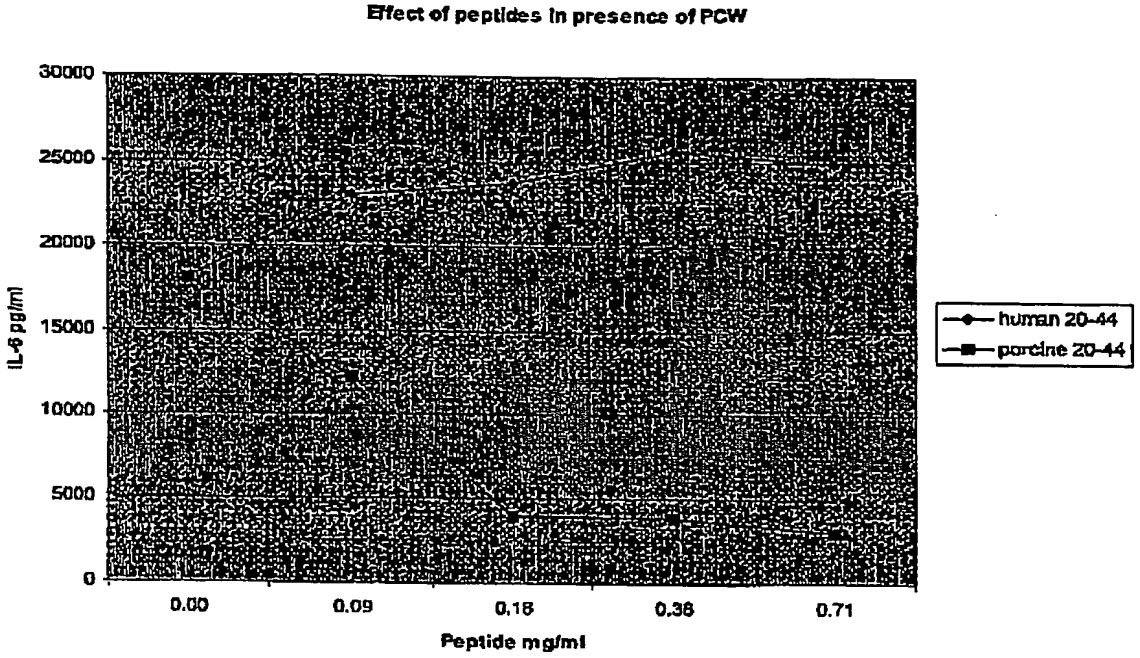
Modtaget
27 JUNI 2003
PVS

Figure 3



Modtaget
27 JUNI 2003
PVS

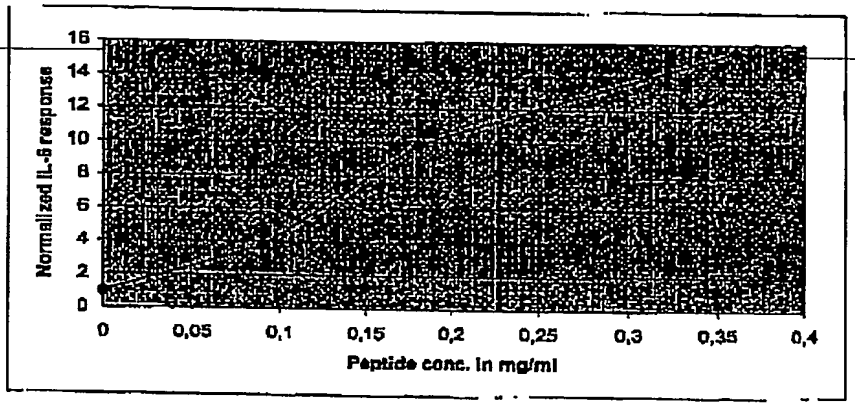
Figure 4



Modtaget
27 JUNI 2003
PVS

Figure 5

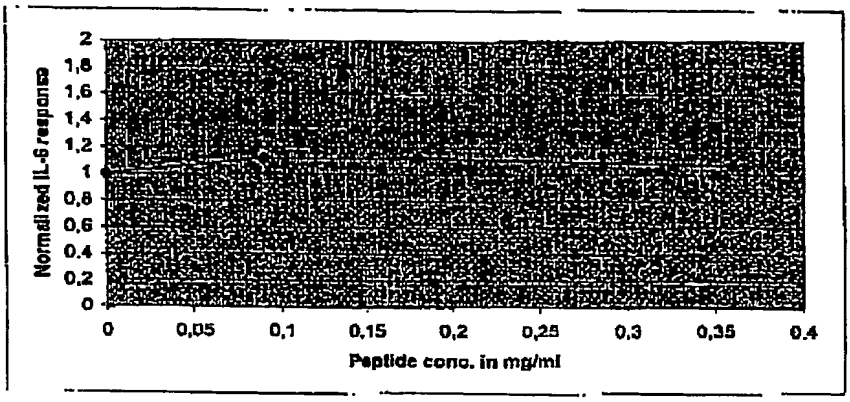
Effect of HBP 20-44 peptides on LPS induced IL-6 secretion in the presence of PCW



Modtaget
27 JUNI 2003
PVS

Figure 6

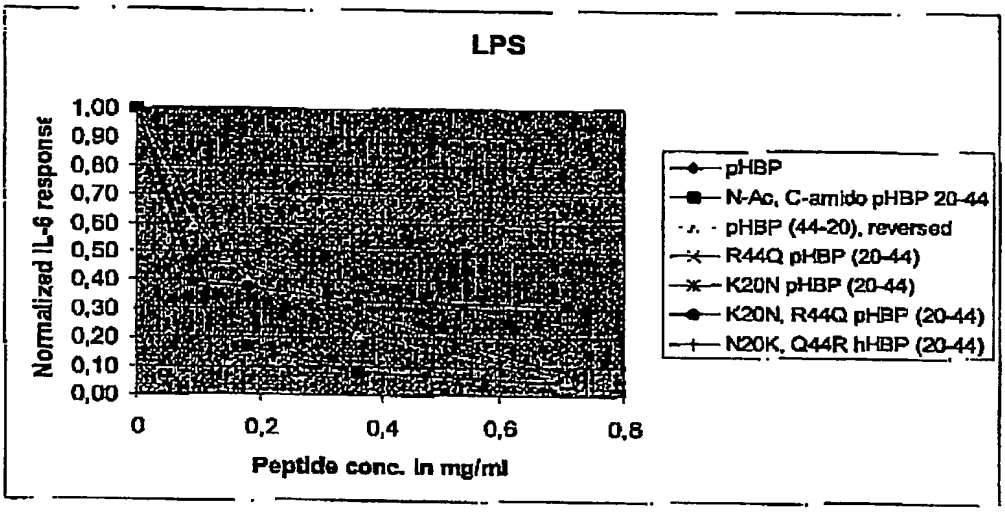
Effect of N-Ac, C-amido hHBP 20-44 on PGN induced IL-6 secretion



Modtaget
27 JUNI 2003
PVS

Figure 7

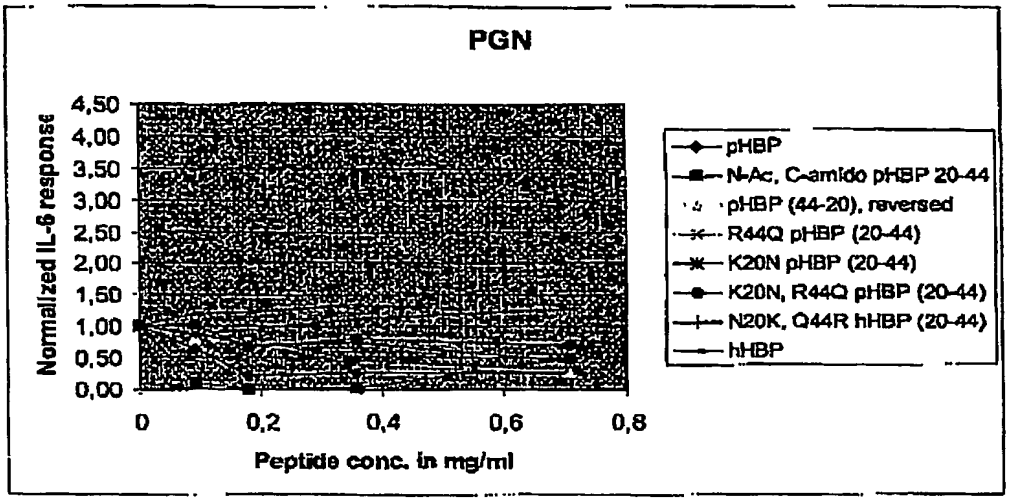
Effect of different substitutions in the sequence of bHBP and p HBP on production of IL-6 induced by LPS.



Modtaget
27 JUNI 2003
PVS

Figure 8

Effect of different substitutions in the sequence of hHBP and p HBP on production of IL-6 induced by PGN



Modtaget
27 JUNI 2003
PVS

Table 1

HBP function	Mono-functional agonist	Mono-functional antagonist
Capillary leakage		Edema, SIRS
Chemotaxis	Wound-healing, local infections	Re-perfusion injury, bran edema
Monocyte activation	Local and systemic infections, (tumors)	Inflammatory diseases, SIRS
Activation contact phase system	(Infection)	SIRS, post-operative bleeding, pain, (inflammation)
Anti-apoptosis	Degenerative diseases	(Tumors)
Endotoxin binding	Endotoxin removal from septic patients	

Potential applications for mono-functional peptides

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☑ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☑ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☑ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.